UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: **June 29, 2015**

SUBJECT: **EDSP Weight of Evidence Conclusions on the Tier 1 Screening Assays for the List 1 Chemicals**

PC Code: See table, Attachment A
Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: See table, Attachment A
MRID No.: NA

DP Barcode: NA
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EPA has completed its Weight of Evidence (WoE) assessment evaluating results of the Endocrine Screening Program (EDSP) Tier 1 screening assays for the List 1 chemicals. The WoE documents for the 52 chemicals are listed in Attachment A along with the chemical and report identifiers.

Attachment A. EDSP List 1 Chemicals

Chemical Name	PC Code	TXR Number
2,4-D	030001	0057151
Abamectin	122804	0057152
Acephate	103301	0057153
Acetone	044101	0057154
Atrazine	080803	0057155
Benfluralin	084301	0057156
Bifenthrin	128825	0057157
Captan	081301	0057158
Carbaryl	056801	0057159
Carbofuran	090601	0057160
Chlorothalonil	081901	0057161
Chlorpyrifos	059101	0057162
Cyfluthrin	128831	0057163
Cypermethrin	109702	0057164
DCPA	078701	0057165
Diazinon	057801	0057166
Dichlobenil	027401	0057167
Dimethoate	035001	0057168
EPTC	041401	0057169
Esfenvalerate	109303	0057170
Ethoprop	041101	0057171
Fenbutatin-Oxide	104601	0057172
Flutolanil	128975	0057173
Folpet	081601	0057174
Glyphosate	417300	0057175
Imidacloprid	129099	0057176
Iprodione	109801	0057177
Isophorone	847401	0057178
Linuron	035506	0057179
Malathion	057701	0057180
Metalaxyl	113501	0057181
Methomyl	090301	0057182
Metolachlor	108801	0057183
Metribuzin	101101	0057184
MGK-264	057001	0057185
Myclobutanil	128857	0057186
Norflurazon	105801	0057150
o-Phenylphenol	064103	0057146
Oxamyl	103801	0057142
PCNB	056502	0057138
Permethrin	109701	0057149
Phosmet	059201	0057145
Piperonyl Butoxide	067501	0057141
Pronamide	101701	0057137
Propargite	097601	0057148
Propiconazole	122101	0057144
Pyriproxyfen	129032	0057140
Simazine	080807	0057136
Tebuconazole	128997	0057143
Tetrachlorvinphos (TCVP)	083701	0057147
Triadimefon	109901	0057139
Trifluralin	036101	0057135

EDSP: WEIGHT OF EVIDENCE ANALYSIS OF POTENTIAL INTERACTION WITH THE ESTROGEN, ANDROGEN OR THYROID PATHWAYS

CHEMICAL: PERMETHRIN

OFFICE OF PESTICIDE PROGRAMS

OFFICE OF SCIENCE COORDINATION AND POLICY

U.S. ENVIRONMENTAL PROTECTION AGENCY

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Abbreviations

Abbreviation	Terminology
A	Androgen (hormonal pathway)
ADME	Absorption, Distribution, Metabolism, Excretion
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AMA	Amphibian Metamorphosis Assay
ARTA	Androgen Receptor Transcriptional Activation
AST	Aspartate Aminotransferase
ANOVA	Analysis of Variance
AOP	Adverse Outcome Pathway
AR	Androgen Receptor
B_{max}	Binding at maximum
BROD	Benzyloxyresorufin-O-dealkylase
BUN	Blood Urea Nitrogen
CAR	Constitutive Androstane Receptor
CFR	Code of Federal Regulations
CG	Cowper's Gland
ChE	Cholinesterase
ChEI	Cholinesterase inhibition
CMC	Carboxymethyl cellulose
CTA	Comparative Thyroid Assay
CV	Coefficient of Variation
CYP	Cytochrome 450
DER	Data Evaluation Record
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DP	Dorsolateral Prostrate
E	Estrogen hormonal pathway
EDSTAC	Endocrine Disruptor Screening and Testing Advisory Committee
EDRT	Endocrine Disruptor Review Team
EDSP	Endocrine Disruptor Screening Program
EE	Ethinyl Estradiol
ELISA	Enzyme Linked Immunosorbent Assay
EOGRTS	Extended One-Generation Reproductive Toxicity Study (Rat)
ER	Estrogen Receptor
EROD	Ethoxyresorufin-O-dealkylase (or deethylase)
ERTA	Estrogen Receptor Transcriptional Activation
EtOH	Ethanol
F	Female
F1	First filial generation

Abbreviation	Terminology
F2	Second filial generation
Fcd	Fecundity
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOB	Field Observation Battery
FQPA	Food Quality Protection Act
Frt	Fertility
FSH	Follicle Stimulating Hormone
FSTRA	Fish Short-Term Reproduction Assay
FT	Flutamide
GD	Gestation Day
GGT	Gamma-glutamyl Transpeptidase
GnRH	Gonadotropin-releasing hormone
GP	Glans Penis
GSI	Gonado-Somatic Index
H	High
HLL	Hind Limb Length
HPG	Hypothalamic-Pituitary-Gonadal Axis
HPLC/MS/MS	High Pressure Liquid Chromatography/Mass Spectroscopy
HPT	Hypothalamic-Pituitary-Thyroidal Axis
I	Inadequate
IC50	Inhibitory Concentration at 50% of response
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative
K_d	Equilibrium Dissociation Constant
K_{ow}	Octanol/Water Partition Coefficient
L	Low dose
LABC	Levator Ani-Bulbocavernosus
LAGDA	Larval Amphibian Growth and Development Assay
LC50	Lethal Concentration in 50% of test organisms
LD	Lactation Day
LH	Luteinizing hormone
LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest Observed Adverse Effect Level
LOQ	Limit of Quantitation
M	Male
MDL	Minimum Detection Level
MEOGRT	Medaka Extended One Generation Reproduction Test
MH	Medium high
ML	Medium low
MoA	Mode of Action
MOE	Margin of Exposure
MRID	Master Record Identifier
MROD	Methoxyresorufin-O-dealkylase
MTC	Maximum Tolerated Concentration

Abbreviation	Terminology
MTD	Maximum Tolerated Dose
N	Negative
NE	Not examined/evaluated
NF stage	Nieuwkoop and Faber's Staging Atlas
NOAEC	No Observed Adverse Effect Concentration
NOAEL	No Observed Adverse Effect Level
NS	Not Statistically Significant
NR	Not Reported
OCSPP	Office of Chemical Safety Pollution and Prevention
OECD	Organization for Economic Co-Operation and Development
OPP	Office of Pesticide Programs
ORD	Office of Research and Development
OSCP	Office of Science Coordination and Policy
OSRI	Other Scientifically Relevant Information
P	Positive
P	Parental generation
PC	Positive Control
PC_{10}	Positive Control at 10% of response
PC_{50}	Positive Control at 50% of response
PND	Post-Natal Day
POD	Point of Departure
PPS	Preputial Separation
PROD	Pentaoxyresorufin-O-dealkylase (or depentylase)
PXR	Pregnane X receptor
QC	Quality Control
RBA	Relative Binding Affinity
RBC	Red Blood Cells
RfD	Reference Dose
RPC_{max}	Relative to Positive Control at maximum
SAP	Scientific Advisory Panel
SC	Solvent Control
s.c	Subcutaneous
SDH	Sorbitol dehydrogenase
SDWA	Safe Drinking Water Act
SEP	Standard Evaluation Procedure
SD	Standard Deviation or Sprague-Dawley
SVL	Snout-to-Vent Length
SV	Seminal Vesicles
T	Thyroid (hormonal pathway)
T1WoERC	EDSP Tier 1 Weight of Evidence Review Committee
T3	Triiodothyronine
T4	Thyroxine (tetraiodothyronine)

Abbreviation	Terminology
TP	Testosterone Propionate
TR	Thyroid Receptor
TSH	Thyroid Stimulating Hormone
UDPGT	Uridine Diphosphate Glucuronyltransferase (also known as UGT)
VC	Vehicle Control
VO	Vaginal Opening
VP	Ventral Prostate
VTG	Vitellogenin
WoE	Weight-of-Evidence

Executive Summary

The Endocrine Disruptor Screening Programs (EDSP) Tier 1 assay battery is designed to provide the necessary empirical data to evaluate the potential of chemicals to interact with the estrogen (E), androgen (A) or thyroid (T) signaling pathways. This interaction includes agonism and antagonism at the estrogen and androgen receptors, altered steroidogenesis, as well as hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary thyroid (HPT) axes. In addition to the available Tier 1 assay data, other scientifically relevant information (OSRI), including general toxicity data and open literature studies of sufficient quality were considered in this weight of evidence (WoE) assessment.

In determining whether permethrin interacts with E, A or T hormone pathways, the number and type of effects induced, the magnitude and pattern of responses observed across studies, taxa and sexes were considered. Additionally, the conditions under which effects occur were considered, in particular, whether or not endocrine-related responses occurred at dose(s) that also resulted in general systemic toxicity or overt toxicity.

On July 23, 2014, the EDS{ Tier 1 Assay Weight of Evidence Review Committee (T1WoERC) of the Office of Pesticide Programs (OPP) and the Office of Science Coordination and Policy (OSCP) conducted a weight-of-evidence (WoE) analysis of the potential interaction of permethrin with the E, A or T hormone pathways. The T1WoERC conclusions from the WoE evaluation in this report are presented by pathway (E, A and then T) beginning with the results of the Tier 1 *in vitro* assays followed by *in vivo* mammalian and wildlife results, then the results of the cited OSRI for mammalian and wildlife studies (40 CFR Part 158 and literature).

For the estrogen pathway, a number of OSRI *in vitro* assays evaluating estrogen receptor activation resulted in conflicting results, ***i.e***. some showed activation of estrogen signaling while others did not. The OSRI assays included estrogen receptor (ER) binding, cellular proliferation of estrogen sensitive MCF-7 cells, expression of estrogen sensitive pS2 mRNA in the cancer cells, and interaction with either ERα or ERβ in transfered HeLa cells. Permethrin was negative in the Tier I *in vitro* aromatase assay, whereas in the steroidogenesis assay permethrin caused an increase ($p<0.05$) in estradiol production. *In vivo*, permethrin was negative in both the uterotrophic and female pubertal rat assays, with no effects on uterine weight, mean age at vaginal opening, percent cycling, or histopathological changes in the ovaries and uterus. In the fish short-term reproduction assay (FSTRA), all effects in females were seen only at a dose that caused overt toxicity (i.e, mortality, clinical signs of toxicity). With the exception of one reproductive study in mallard duck which had a slight reduction in the number of eggs laid by the hen (which has limited ability to inform about potential estrogen-related effects by itself and decreases in food consumption also observed at this concentration), no estrogen-related effects were noted in the *in vivo* studies in mammals, fish, or birds that occurred in the absence of overt

and/or systemic toxicity. Therefore, although several of the *in vitro* studies [estrogen receptor (ER) binding, ER transactivation assay (ERTA), and steroidogenesis] indicated that permethrin had the potential to affect the E pathway at the cellular level, these effects are not supported by the available *in vivo* studies.

For the androgen pathway, conflicting results were reported for AR interaction across the *in vitro* assays. In an *in vitro* assay, permethrin reduced sperm motility in a concentration-dependent manner (1-64 µmol/L). Multiple Hershberger assays were available for permethrin, in which the Tier 1 assays as well as an additional open literature study (Kunimatsu, *et al*., 2002) were negative at doses up to 75 or 120 mg/kg/day, respectively. However, in another OSRI Hershberger assay (Kim *et al*., 2005), significant decreases in AST weights were reported at doses of ≥10 mg/kg/day.

In the male rat pubertal assay, no androgen-related effects were observed. While there does not appear to be androgen-related effects in rats with an intact HPG axis (i.e. male pubertal and Part 158 rat data); published results in the literature (Jin *et al*., 2012, Wang *et al*., 2012) reported that permethrin caused testicular toxicity in mice following oral exposure at a dose of 35 or 100 mg/kg/day. In these studies, testicular toxicity manifested as changes in testosterone levels, decreased testicular and epididymal weights, changes to the epididymal duct, regression of sperm quality and histopathological lesions in the testes. In the FSTRA, all effects in males were only observed at the high concentration in the presence of overt toxicity.

Therefore based on the available *in vitro* and mammalian *in vivo* data, there appears to be a potential interaction with the androgen pathway in mammals. There was no convincing evidence of a potential interaction with the androgen pathway in wildlife.

There was no convincing evidence of an interaction of permethrin with the thyroid pathway in mammals or wildlife in the absence of overt toxicity. The only thyroid-related effect observed in the absence of overt toxicity was a decrease in serum T4 levels in the male and female pubertal assays. No thyroid related effects were observed in the mammalian Part 158 studies. No treatment-related thyroid effects were seen in the AMA.

Overall, there is no convincing evidence of potential interaction with the estrogen or thyroid pathways in mammals or wildlife. There is evidence for potential interaction with the androgen pathway in mammals. There is no convincing evidence for a potential interaction with the androgen pathway in wildlife.

For permethrin, a point of departure (POD) of 25 mg/kg/day for human health risk assessment is based on neurotoxic clinical signs (abnormal and/or decreased movement, increased body temperature, and aggression) observed at 75 mg/kg/day (LOAEL) in an acute neurotoxicity study in rats. This POD is used for deriving the chronic Reference Dose (RfD) for chronic dietary risk

assessment. A second POD of 11 mg/kg/day based on clinical signs of toxicity (body temperature and hypersensitivity to noise) seen at 154 mg/kg/day (LOAEL) in a subchronic inhalation toxicity study in rats. This POD is used for calculating the Margins of Exposure (MOEs) for non-dietary risk assessment. These PODs are lower than the dose (100 mg/kg/day) that caused the testicular effects in mice. Consequently, the current RfD and MOEs are protective of the androgen-mediate effects observed in the published literature study. Additionally, there was no evidence of interaction with the androgen pathway in the EDSP Tier 1 assays. Therefore, there is no concerns for the findings reported in the literature study.

Based on weight of evidence considerations, mammalian EDSP Tier 2 testing is not recommended for permethrin since additional testing is not expected to impact EPA's current regulatory point of departures and endpoints for human health risk assessments.

Given the lack of evidence for a potential interaction with the estrogen and androgen pathway in fish and thyroid pathway in amphibians, no additional testing is recommended.

For birds, Part 158 avian reproduction studies with both northern bobwhite quail and mallard duck are available for permethrin. In the bobwhite quail, no reproductive effects were observed, but effects on reproductive parameters were observed in the mallard duck. The type of data obtained from Part 158 avian reproduction studies (OCSPP 850.2300) are considered sufficient for evaluating potential reproductive effects to birds from permethrin exposure. Relative to the EDSP, additional testing is not recommended.

I. Introduction

The Endocrine Disruptor Screening Programs (EDSP) Tier 1 assay battery is designed to provide the necessary empirical data to evaluate the potential of chemicals to interact with the estrogen (E), androgen (A) or thyroid (T) signaling pathways. This interaction includes agonism and antagonism at the estrogen and androgen receptors, altered steroidogenesis, as well as hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary thyroid (HPT) axes. In addition to the available Tier 1 assay data, other scientifically relevant information (OSRI), including general toxicity data and open literature studies of sufficient quality were considered in this weight of evidence (WoE) assessment.

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Permethrin has a water solubility of <1 mg/L, and a vapor pressure of is 2.15×10^{-8} mm Hg (25°C). Permethrin was immobile in several soils tested (Koc >5000). It is also slow to hydrolyze and biodegrade (anaerobic aquatic study half-lives of 113-175 days). Based upon its Henry's law constant (1.4×10^{-6} atm-m^3/mol) and vapor pressure, permethrin is expected to have a relatively low potential for volatilization from soil and water surfaces. The potential of permethrin for volatilization is also reduced significantly because it adsorbs strongly to soils and suspended solids or sediment in the water column.

The available information considered in determining the potential interaction of permethrin with the E, A, or T pathways include submitted EDSP Tier 1 assays and/or other scientifically relevant information (OSRI) such as general toxicity studies and other published articles. These data are summarized in Sections III.A through III.C. An analysis of the data submitted to the agency, using the WoE approach outlined by the Agency (USEPA, 2011), is presented in Section IV. The EDSP Tier 2 study recommendations are presented in Section V.

II. Sources of Scientific Data and Technical Information

A. EDSP Tier 1 Screening Assays

The Tier 1 assays and/or other scientifically relevant information (OSRI) submitted to satisfy the agency's test order are shown below in Table 1. Executive Summaries are presented in Appendix 1.

B. Other Scientifically Relevant Information (OSRI)

In response to the Agency's Test Orders, data believed to be relevant to one or more of the Tier 1 Screening assays were submitted as OSRI by the Test Order recipients and/or the public. This included studies published in the open literature and/or data submitted to support pesticide registration (e.g., Part 158 guideline studies). The Agency's review of the initial OSRI is provided in the Report of the Endocrine Disruptor Review Team on permethrin (USEPA, 2010). Since then, the Agency has also conducted a more recent search of available scientific literature for any additional relevant information. Summaries of the available OSRI are presented in Appendix 2. Additionally, literature/studies considered but not utilized for the WoE analysis are listed in Appendix 3.

III. Weight of Evidence (WoE) Evaluation

The principles, criteria and approach used in the WoE determination on the potential of a substance to interact with endocrine-related processes (*i.e.,* E, A, or T hormone pathways) were as described in the WoE guidance document (USEPA, 2011) and presented at the 2013 FIFRA Scientific Advisory Panel (SAP) (USEPA, 2013). The weight of evidence process identifies how the individual lines of evidence are assembled and integrated along two concepts (*i.e.*, complementarity and redundancy) within the conceptual framework of an adverse outcome pathway). Broadly, there are four main steps outlined in the guidance which provide the foundation for WoE evaluations. The first step is to evaluate the individual studies for their scientific quality and relevance in evaluating potential endocrine interaction(s). The second step is to integrate the data along different levels of biological organization while examining the extent of complementarity (*i.e*., the concordance of endpoints within an assay that measures multiple endpoints) and redundancy (*i.e*., the concordance of endpoints/responses across assays) in the observed responses across these different levels of biological organization. The third step is to characterize the main lines of evidence as well any conclusions. Finally, the last step is to evaluate whether additional testing is needed based on the evidence and conclusions described above.

As mentioned, the first step is to assemble and evaluate the available scientific data. Data for the EDSP Tier 1 WoE evaluation falls into one of two categories: 1) EDSP Tier 1 data, or 2) other scientifically relevant information (OSRI). The EDSP Tier 1 data includes a battery of 11 assays consisting of *in vitro* and mammalian and wildlife *in vivo* assays. The Tier 1 assays were designed specifically to evaluate a number of key biological events including potential effects on receptor binding (estrogen and androgen agonist and antagonist), steroidogenesis, and other effects on the HPG and HPT axes. OSRI may include published literature studies as well as studies conducted under USEPA (often referred to as Part 158 data) or OECD guidelines submitted in support of pesticide registrations. Each study is evaluated for scientific quality and relevance for informing interactions with the E, A, or T pathway. Additionally, the consistency of the responses in the individual study is evaluated. For the Tier 1 *in vivo* assays, often multiple endpoints are measured in each assay.

Evaluation of the potential confounding effects of overt toxicity in the study, as well as the relative degree of diagnostic utility of a specific endpoint for discerning whether or not the chemical has interacted with the endocrine system, are considered. The collective response of the individual endpoints, as well as the conditions under which they were expressed, are considered when evaluating an overall indication of potential interaction as measured by the study.

The second step in this WoE process is to formulate hypotheses and integrate the available data along different levels of biological organization. Two keys elements in the integration of data, as well as characterizing the extent to which the available data support a hypothesis that a chemical has the potential to interact E, A or T pathways, are the concepts of complementarity and redundancy. These two concepts provide a basis for considering the plausibility, coherence, strength, and consistency of the body of evidence. The current EDSP Tier 1 screening assays are meant to evaluate whether or not a chemical can interact with E, A, and T consisting of different levels of biological organization from a molecular initiating event, such as receptor binding, through potential adverse effects in apical endpoints such as sexual development and fecundity at the whole organism level. The extent of expectation of responses at higher levels of biological organization can indirectly provide information on potential compensatory capabilities of an individual organism.

After the data have been assembled and integrated, the third step is to characterize the main lines of evidence along with the conclusions; this characterization involves three components. The first component is whether the data provide relevant, robust and consistent evidence in terms of complementarity and redundancy, as well as biological plausibility. Second, is at what level of biological organization were the responses observed and whether organisms exhibit compensatory responses at higher levels of biological organization?. Finally, under what conditions did the responses occur including consideration of whether the responses were observed in the presence of overt or systemic toxicity?. The presence of overt and/or systemic

toxicity introduces uncertainty in the ability to distinguish effects specifically related to an endocrine-related effect from a non-endocrine toxic response.

This uncertainty in distinguishing whether the responses were endocrine-related was discussed at the FIFRA SAP meeting in that evaluated scientific issues associated with the WoE evaluation of the EDSP Tier 1 screening process. In October, 2013 the SAP stated that , "*In summary, the Panel agreed that little, if any, weight should be placed on signs of endocrine disruption in the presence of overt toxicity. All effects in endocrine sensitive tissues should be evaluated in terms of primary interactions with the endocrine system vs. secondary effects related to toxicity in non-endocrine organs or overall disruptions in homeostasis"* (USEPA, 2013).

For these WoE analyses, overt toxicity was generally defined in accordance with EPA's current approach as used by OPP in reviewing 40 CFR Part 158 studies for both human and ecological risk assessments. Specifically, in these analyses, the effects that EPA considered to be potential evidence of overt toxicity included, but were not limited to: mortality; clinical signs such as tremors, ataxia and abnormal swimming (fish and aquatic-phase amphibians); and body weight decreases of ≥10% in mammals. Additionally, other effects including morphological (*e.g.*, organ weights/histopathology), biochemical (*e.g.*, blood chemistry), and other clinical signs (*e.g.*, lethargy) were also considered when evaluating overt toxicity, especially if the effects were extreme. In some instances, one parameter (*i.e.*, death or >10% decrease in mammalian body weight) was sufficient to consider a dose/concentration to be overtly toxic. However, in other instances, more than one parameter was needed to determine overt toxicity. For example, in the FSTRA, generally, body weight decreases were considered along with other responses when assessing potential overt toxicity. Additionally, effects which were considered to be signs of systemic toxicity were also captured and these effects were generally considered as less severe forms of toxicity (*e.g.*, change in organ weights or blood chemistry). The circumstances for which a dose/concentration was considered overtly toxic for a particular study are described in Section IV.A.

Therefore, EPA considers multiple lines of evidence in including the observed responses in the Tier 1 assays and OSRI in the context of a chemical's physical/chemical properties and its known modes of action in its overall characterization of a chemical's potential to interact with the E, A or T pathway. Adequately addressing the three main components described above is fundamental to the WoE process and in determining whether additional data are needed. In addition to characterizing the WoE, reviewers also considered: 1) uncertainties and their potential impact to conclusions; 2) discussion of key studies; 3) description of inconsistent or conflicting data; 4) overall strength of evidence supporting a conclusion; and, 5) what, if any, additional data are needed and why. Assessing the need for additional data is based on a case-by-case analysis which took all available toxicity data into account.

The WoE approach involved consideration of data (i.e., lines of evidence) from the EDSP Tier 1 assays and OSRI which are depicted in **Tables 2 - 4**. *These tables contain data that are considered scientifically and biologically relevant with regard to a treatment-related effect which supports a conclusion of whether a substance has the potential to interact with the E, A, or T pathway. Effects that occurred in the presence of overt toxicity are discussed in the text for each respective pathway (E, A or T) but are not reported in the table for E, A or T.*

A. EDSP T1 Screening Assays

The Tier 1 assays submitted in response to the agency's test order for permethrin are shown below in **Table 1**.

Table 1. Tier 1 Screening Assays for Permethrin

Tier 1 Assays	Test Guideline	Test Order Status
ER Binding Assay (Rat uterine cytosol)	OSCPP 890.1250	Requirement Satisfied by OSRI (Saito *et. al.*, 2000)
ERα Transcriptional Activation Assay (Human cell line HeLa 9903)	OSCPP 890.1300	Requirement Satisfied by OSRI (Tyler *et. al.*, 2000)
AR Binding Assay (Rat prostate cytosol)	OSCPP 890.1150	Requirement Satisfied by OSRI (Xu *et. al.*, 2008; Zhang *et. al.*, 2008a; Tyler *et. al.*, 2000; Zhang *et. al.*, 2008b; Kim *et. al.*, 2005)
Steroidogenesis Assay (Human cell line H295R)	OSCPP 890.1550	Requirement Satisfied (MRID No. 48618603)
Aromatase Assay (human recombinant microsomes)	OSCPP 890.1200	Requirement Satisfied (MRID No. 48618601)
Uterotrophic Assay (Rat)	OSCPP 890.1600	Requirement Satisfied by OSRI (Kim *et. al.*, 2005; Kunimatsu *et. al.*, 2002)
Hershberger Assay (Rat)	OSCPP 890.1400	Requirement Satisfied (MRID No. 48618602)
Pubertal Female Assay (Rat)	OSCPP 890.1450	Requirement Satisfied (MRID No. 48672901)
Pubertal Male Assay (Rat)	OSCPP 890.1500	Requirement Satisfied (MRID No. 48687101)
Fish Short-term Reproduction Assay	OSCPP 890.1350	Requirement Satisfied (MRID No. 48702301)
Amphibian Metamorphosis Assay (Frog)	OSCPP 890.1100	Requirement Satisfied (MRID No. 48988601)

B. Effects on Hypothalamic-Pituitary-Gonadal (HPG) Axis

1. Effects on Estrogen Pathway

The potential interactions of permethrin with the estrogen pathway are summarized in **Table 2.** The various targets of the estrogen pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for estrogenic, anti-estrogenic, or HPG axis effects. This table also includes HPG-relevant findings from data evaluated as OSRI. *Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.*

The requirement for the Tier 1 ER binding and ERTA assays were satisfied by Saito *et al*., 2000 and Tyler *et al*., 2000, respectively. In the study by Saito *et al*., permethrin was negative for interaction with the ER based on the results of three *in vitro* assays utilizing human ER (hERα): 1) a mammalian cell-based luciferase reporter assay, 2) a yeast two-hybrid method, and 3) a competitive ligand-binding assay. Conversely, in the study by Tyler *et al*., permethrin showed weak activity in a recombinant yeast reporter assay with a half maximal effective concentration (EC_{50}) of 2×10^{-3} M. In other published studies considered as OSRI, the effects of permethrin in ER binding, ER transcriptional activation, cell proliferation, and ER-responsive pS2 gene expression showed conflicting results regarding the estrogenic potential of permethrin. The findings from the additional *in vitro* OSRI considered to evaluate potential ER activity are summarized below.

In an ER competitive-binding study using rat uterine cytosol (Chen *et al*., 2002), permethrin exhibited equivocal evidence of specific binding to the ER. The percent [^{3}H]-E_2 binding to the ER was ≥ 67% at all concentrations up to 10^{-3} M permethrin. However, in another ER competitive binding assay also using rat uterine cytosol, Kim *et al*. (2004) reported that permethrin did not competitively bind to the ER, while in a whole cell competitive binding study, Lemaire *et al*. (2006), reported that permethrin did not bind to ERα or ERβ.

Permethrin was also evaluated in several ER transactivation test systems. In a study by Du *et al*. (2010), permethrin was tested for potential interaction with the ERα using an ER transactivation assay in African green monkey CV-1 cells. Permethrin exhibited weak agonist effects at low concentrations (10^{-8} to 10^{-5} M), and exhibited weak antagonist effects at higher concentrations (10^{-6} to 10^{-5} M). In a study by Lemaire *et al*. (2006), permethrin was evaluated for ERα and ERβ activity in transfected HeLa-derived (HELN) cells. In this study, the authors reported that permethrin showed no agonist or antagonist activity for ERα or ERβ at the single concentration (10^{-5} M) tested.

Published studies also showed conflicting results regarding the ability of permethrin to induce ER-responsive cell proliferation. In a MCF-7 cell proliferation assay by Chen *et al*. (2002), permethrin induced proliferation at and above 10^{-10} M, with maximal proliferation at 10^{-8} M. In a study by Go *et al*. (1999), permethrin induced modest increases in MCF-7 proliferation at 10^{-4} M and in a study Jin *et al*. (2010), permethrin induced proliferation of MCF-7 cells at concentrations of 10^{-8} to 10^{-6} M. Conversely, in a study by Kim *et al*. (2004), permethrin did not induce proliferation in MCF-7 cells, but it did antagonize the proliferative response of estradiol at 10^{-6} M.

Several of the published studies also measured pS2 mRNA levels as a biomarker for estrogenic activity in MCF-7 cells. The pS2 gene is strongly expressed in response to estrogen or estrogen-like compounds. In a study by Chen *et al*. (2002), pS2 mRNA expression increased 1.3-fold after treatment with 10^{-7} M permethrin. Go *et al*. (1999), reported permethrin weakly induced pS2 expression at 10^{-4} M and Jin *et al*. (2010) reported that permethrin induced pS2 expression 2.6-fold and decreased ERα mRNA expression levels at 10^{-6} M.

In another *in vitro* study, Garey and Wolf (1998) evaluated the potential for permethrin to act as an estrogen agonist or antagonist using the Ishikawa Var-1 human endometrial adenocarcinoma cell line. This cell line produces a dose-dependent increase in alkaline phosphatase in response to estrogens. Permethrin did not show estrogen agonist or antagonist activity in this test system.

Permethrin was negative in the Tier 1 aromatase assay; while in the Tier 1 steroidogenesis assay, permethrin produced an increase ($p<0.05$) in estradiol production in two runs at 1 and 10 µM (average 1.16-fold for 1 µM and 1.51-fold for 10 µM).

The test order requirement for the uterotrophic assay was satisfied by the results of two literature studies submitted as OSRI: Kim *et al*., 2005; and Kunimatsu *et al*., 2002. In Kim *et al*., permethrin (administered s.c.) increased relative (to body) uterus weights at a dose level of 800 mg/kg/day. Permethrin also enhanced E_2-induced uterine weight increases at a lower dose (200 mg/kg); permethrin-induced uterine weight increases were inhibited by co-administration of the anti-estrogen ICI 182,780, but inhibition was only statistically different at a lower dose (50 mg/kg). Kim also reported that permethrin treatment had no effects on relative vagina weights. In Kunimatsu *et al*., no estrogenic or anti-estrogenic effects were observed on uterine weights with oral administration up to 150 mg/kg/day permethrin which also caused clinical signs of toxicity (3-6/6 rats displaying tremor). No estrogen-related effects were observed in the Tier 1 female pubertal assay.

In the Tier 1 FSTRA, increased GSI and effects on gonadal histopathology along with a 42% decrease in fecundity were observed at the highest concentration tested. However, overt toxicity (*i.e.,* mortality, clinical signs of toxicity) was noted at this dose level.

In a Part 158 developmental toxicity study in rabbits, post-implantation loss was increased ($p<0.05$) at the mid (1200 ppm) and high (1800 ppm) dose levels; however, rabbits in all dose groups (including 600 ppm) had decreases in body weight gains of 50-91% during GD 0-18. There were no estrogen-related effects noted in the other Part 158 studies.

In a Part 158 avian reproduction toxicity study with quail, no treatment-related systemic or reproductive effects were noted at dietary levels up to 500 ppm. In the mallard duck reproduction study, there appeared to be a slight reduction (NS, but considered biologically significant) in the number of eggs laid by hens at the high dose (500 ppm) during the last two weeks of egg production, which was correlated with an increase in the number of hens with a regressing ovary. However, feed consumption at this dose was observed to be slightly decreased throughout the study, with a significant difference from controls during Weeks 6, 12, 14, and 19.

In a Part 158 fish life-cycle toxicity study with fathead minnows, at the mean measured concentration 0.41 ppb, percent survival of fry were reduced in the first and second generations after 30 days. In a study by Nillos *et al*. (2010), permethrin stereoisomers were examined for estrogenic activity *in vivo* using VTG protein measurements in male medaka. After exposure of medaka for 8 days to the individual permethrin stereoisomers and permethrin mixture at 10 µg/L, hepatic VTG production was increased ($p<0.05$) compared to controls. Additionally, significant differences in the relative estrogenic potential of the enantiomers were reported: 1S-cis- and 1S-trans-permethrin had responses 2.5 and 1.3 times greater than their respective R enantiomers. However, this study was conducted under static conditions and as such, given the physical properties of permethrin (low solubility, propensity to bind), there is concern about the actual exposure concentrations (study not included in table below).

In Brander *et al*., 2012, silverside fish (*Menidia beryllina*) exposed for 14 days to 0.1 ng/L of bifenthrin exhibited significantly greater choriogenin (precursor to chorion, which is an estrogen dependent protein that forms the layer between the embryo and the outer layer of the egg) expression than 1 ng/L of the positive control, ethinylestradiol. It is worth noting that this study did not have a negative control but rather comparisons were made to a methanol solvent control. Additionally, the measured test concentrations reported appear to reflect results from a single 24 hour renewal cycle under abiotic (*i.e.* without fish) conditions. Since, these results are confounded by several uncertainties including the absence of a negative control and limited exposure measurements, and this study is not reported in the table below.

In Jin et al. 2009, zerbrafish embryo-larvae were exposed to permethrin for 7 days under static-renewal conditions exhibited upregulation of several estrogen-responsive genes (e.g., vtg1, esrα, cyp19b) at 250 ng/L and above. In this study, as in other studies, only a solvent control was used and given the static-renewal test design without reported measured test concentrations, there is uncertainty in the actual exposure concentrations. As such, this study is not reported in table below.

Table 2: Estrogenic/Anti-Estrogenic Pathway for Permethrin.

Lines of Evidence Indicating Potential Interaction with the Estrogenic/Anti-Estrogenic Pathway for Permethrin[1]															
Study Type/ Literature Citation	ER Binding	ER Activation	Steroidogenesis	Sex Steroid Hormones	Uterine Weight	Ovarian Weight/GSI	Ovarian/Gonad Staging and Histopathology	Pituitary Weight	Estrous Cyclicity	Age & Weight at VO	2° Sex Characteristics	Fertility (Frt)/ Fecundity (Fcd)	Vitellogenin	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
EDSP Tier 1 Assay															
ER Binding	Requirement satisfied by OSRI (Saito *et al*., 2000); see below.														
ERTA	Requirement satisfied by OSRI (Tyler *et al*., 2000); see below.														
Aromatase (MRID 48618601)			N												
Steroidogenesis (MRID 48618603)			P												
Uterotrophic	Requirement satisfied by OSRI (Kim *et al*., 2005; Kunimatsu *et al*., 2002); see below.														
Female Pubertal Rat (MRID 48672901)					N	N	N	N	N	N				↑LW (H)	X (H)
FSTRA (MRID 48702301)				NE		N	N				N	N	N		X (H)
OSRI															
ER Binding and Transactivation (Saito *et al.*, 2000)	N	N													
Recombinant Yeast Transactivation Assay (Tyler *et al.*, 2000)		P													
ER Binding/ Proliferation/PS2 (Chen *et al.*, 2002)	E	P[4]													
ER Binding/ Proliferation/PS2 (Kim *et al.*, 2004)	N	P[4]													

Table 2: Estrogenic/Anti-Estrogenic Pathway for Permethrin.

Lines of Evidence Indicating Potential Interaction with the Estrogenic/Anti-Estrogenic Pathway for Permethrin[1]															
Study Type/ Literature Citation	ER Binding	ER Activation	Steroidogenesis	Sex Steroid Hormones	Uterine Weight	Ovarian Weight/GSI	Ovarian/Gonad Staging and Histopathology	Pituitary Weight	Estrous Cyclicity	Age & Weight at VO	2° Sex Characteristics	Fertility (Frt)/ Fecundity (Fcd)	Vitellogenin	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
ER Binding/ ER Transactivation (Lemaire *et al.*, 2006)	N	N[5]													
ER Transactivation (Du *et al.*, 2010)		P[6]													
Proliferation/PS2 (Jin *et al.*, 2010)		P[4]													
Proliferation/PS2 (Go *et al.*, 1999)		P[4]													
Estrogenic Potential (Garey and Wolff, 1998)		N[7]													
Uterotrophic (Kim *et al.*, 2005)					N									N	N
Uterotrophic (Kunimatsu *et al.*, 2002)					N									X (H)	N
Three-Generation Reproduction (Rat; MRID 00120271)					NE	NE	N	NE	NE			N		X (H)	X (H)
Developmental Toxicity (Rat; MRID 40943603)												N		X (H)	X (H)
Developmental Toxicity (Rabbit; MRID 40943602)												P (M)		X (L, M, H)	X (H)
Chronic Toxicity/ Carcinogenicity (Rat; MRID 00069701)					NE	N	N	N						X (H)	X (H)
Chronic Toxicity (Dog; MRID 00129600)					NE	N	N	N						X (M, H)	X (H)

Table 2: Estrogenic/Anti-Estrogenic Pathway for Permethrin.

Lines of Evidence Indicating Potential Interaction with the Estrogenic/Anti-Estrogenic Pathway for Permethrin[1]															
Study Type/ Literature Citation	ER Binding	ER Activation	Steroidogenesis	Sex Steroid Hormones	Uterine Weight	Ovarian Weight/GSI	Ovarian/Gonad Staging and Histopathology	Pituitary Weight	Estrous Cyclicity	Age & Weight at VO	2° Sex Characteristics	Fertility (Frt)/ Fecundity (Fcd)	Vitellogenin	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
28-Day Feeding (Rat; MRID 00120267)					NE	N	N	N						X (MH, H)	X (H)
Avian Reproduction (Quail; MRID 2322901)												N		N	N
Avian Reproduction (Duck; MRID 2322902)												P[8]		N	N[9]
Fish Full Life Cycle (Minnow; MRID 00102096)												N			X (MH, H)

1. Key to responses: L=Low treatment, ML=Medium-low treatment, M=Medium treatment, MH=Medium-high treatment, H=High treatment Arrows (↓ or ↑) indicate the direction of the response. A shaded cell indicates that is parameter is not routinely evaluated or is not applicable in this assay. Changes in weight are absolute unless otherwise indicated. LW= Liver weight
2. The systemic toxicity in the Tier 1 assays are presented in this column (*e.g.* KW= kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
3. The overt toxicity in the Tier 1 assays are presented in this column (*e.g.* ↓BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
4. Reported increased MCF-7 proliferation and induction of PS2 expression.
5. Negative at the single permethrin concentration tested (10^{-5} M)
6. Permethrin exhibited weak agonist effects at low concentrations (10^{-8} to 10^{-5} M), and exhibited weak antagonist effects at higher concentrations (10^{-6} to 10^{-5} M) in CV-1 cells.
7. Negative for estrogen agonist or antagonist using the Ishikawa Var-1 human endometrial adenocarcinoma cell line test system.
8. Slight reduction (NS, but considered biologically significant) in the number of eggs laid by hens at the high dose (500 ppm) during the last two weeks of egg production, which was correlated with an increase in the number of hens with a regressing ovary.
9. Feed consumption at this dose was slightly decreased throughout the study, with a significant difference from controls during Weeks 6, 12, 14, and 19.

E Equivocal
P Positive findings
N Negative findings (*in vitro*)/ No effects (*in vivo*)
NE Not Examined

2. Effects on Androgen Pathway

The potential interactions of permethrin with the androgen pathway are summarized in **Table 3.** The various targets of the androgen pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for androgenic, anti-androgenic, or HPG axis effects. This table also includes HPG-relevant findings from data evaluated as OSRI. This table also includes HPG-relevant findings from data evaluated as OSRI. *Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.*

The requirement for the AR binding assay was satisfied by the results of four studies submitted as OSRI: Xu *et al.,* 2008; Zhang *et al.,* 2008; Tyler *et al.*, 2000; and Kim *et al.,* 2005. In an *in vitro* AR transcriptional activation assay Xu *et al*., reported that permethrin showed AR antagonist activity by attenuating dihydrotestosterone (DHT)-related chloramphenicol transferase (reporter gene) expression with an IC_{50} value of 5.86×10^{-5} M. In Zhang *et al*., 2008, permethrin significantly inhibited DHT-induced hAR transcriptional activation *in vitro* at a concentration of 10^{-5} M. In Tyler *et al*., high concentrations of permethrin ($\sim 10^{-3}$ M) induced slight increases in activity above baseline values, thus suggesting very weak agonist activity. Permethrin also exhibited weak anti-androgenic activity in this assay with an IC_{50} of 7.3×10^{-4} M. Additionally, two published studies examining the effects of permethrin on AR binding and/or AR transactivation were considered as OSRI. In a study by Bauer *et al*. (2002), permethrin did not displace DHT from immobilized recombinant hAR. Conversely, in a receptor transactivation study by Du *et al*. (2010), permethrin showed AR antagonist activity with a calculated 20% relative inhibitory concentration (RIC_{20}) of 1.88×10^{-6} M. In the Tier 1 *in vitro* steroidogenesis assay, permethrin induced a slight, but significant ($p<0.05$) increase in testosterone production at 10 µM in two runs (average 1.1-fold).

Permethrin was negative in the Tier 1 Hershberger assay and no androgen related effects were noted in the Tier 1 male pubertal assay. Additionally, in a published 5-day Hershberger assay by Kunimatsu *et al*. submitted as OSRI, no effects were noted on the accessory sex tissues at dose levels that caused tremors (75 mg/kg/day). In another published 10-day Hershberger assay, Kim *et al*. (2005) reported statistically significant reductions in the five sex accessory tissue weights at all doses tested (10, 50, and 100 mg/kg/day), although the decreases were not dose dependent. In addition, permethrin caused significant decreases in seminal vesicle and ventral prostate weights in a modified Hershberger assay conducted by Zhang *et al*. (2008) using a single dose level (50 mg/kg/day) of permethrin; however, no chemical purity information was provided which reduces confidence in the study and therefore the results are not included in the table below.

In the Tier 1 FSTRA, effects were noted in males at the highest concentration tested (2.6 µg a.i./L), including an increase in the GSI and decreased nuptial tubercle score. However, only 63% of the males survived at the high dose, and clinical sings of toxicity were noted at this dose, including erratic swimming, loss of equilibrium, surfacing, and excess feed noted in the test chambers. There were no androgen-related effects determined in the Part 158 ecotoxicity studies.

The only androgen-related effect in the Part 158 mammalian studies was decreased testicular weight seen in a rat 28-day feeding toxicity study at doses that also caused neurotoxicity (hypersensitivity).

In addition to the Part 158 toxicity studies, published studies were reviewed that specifically examined effects of permethrin on testes and/or sperm parameters in rodents. In a testicular toxicity study by Jin *et al*., (2012), (+)-cis, (−)-cis, and (−)-trans permethrin exposure at 100 mg/kg significantly reduced serum testosterone levels, and decreased both absolute and relative weights of testes in the (+)-cis, (−)-cis and (−)-trans-permethrin treated groups. A decreased number of spermatogenic cells and large interstitial spaces in the seminiferous tubules were also observed in the 100 mg/kg (+)-cis, (−)-cis, and (−)-trans-permethrin treated groups. In a testicular toxicity study by Wang *et al*. (2012), the percentage of seminiferous tubules in stage group VII-VIII was significantly decreased in the cis-permethrin group (oral dosing at ~35 mg/kg/day), and in the caput epididymis, cytoplasmic vacuolation was observed more frequently in the cis-permethrin group, and median vacuolation scores were significantly higher. Caudal epididymal sperm count was significantly reduced in the cis-permethrin group (↓60%), and sperm motility was significantly decreased.

Table 3: Androgenic/Anti-Androgenic Pathway for Permethrin.

Lines of Evidence Indicating Potential Interaction with the Androgenic/Anti-Androgenic Pathway for Permethrin[1]															
Study Type/ Literature Citation	AR Binding	AR Activation	Steroidogenesis	Sex Steroid Hormones	Testes Weight/GSI	Gonad Staging and Histopathology	Epididymides Weight	Epididymides Histopathology	Pituitary Weight	Accessory Sex Organ Weights/ 2° Sex Characteristics	Fertility (Frt)/ Fecundity (Fcd)	Age and Weight at PPS	Vitellogenin	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
EDSP Tier 1 Assay															
AR Binding	Requirement satisfied by OSRI (Xu *et al.*, 2008; Zhang *et al.*, 2008a; Tyler *et al.*, 2000; Zhang *et al.*, 2008b; Kim *et al.*, 2005)														
Steroidogenesis (MRID 48618603)			P												
Hershberger (MRID 48618602)				NE						N				N	N
Male Pubertal Rat (MRID 486687101)				N	N	N	N	N	N	N		N		↑LW (L, H)	X (H)
FSTRA (MRID 48702301)				NE	N	N				N	N		N		X (H)
OSRI															
AR Binding (Bauer *et al.*, 2002)	N														
Recombinant Yeast AR Assay (Tyler *et al.*, 2000)		P													
Receptor Transactivation (Du *et al.*, 2010)		P													
AR Transactivation (Xu *et al.*, 2008)		P													
Hershberger (Kim *et al.*, 2005)				NE						P (L, M, H)				N	N
Hershberger (Kunimatsu *et al.*, 2002)				NE						N				N	X (H)

Table 3: Androgenic/Anti-Androgenic Pathway for Permethrin.

Lines of Evidence Indicating Potential Interaction with the Androgenic/Anti-Androgenic Pathway for Permethrin[1]															
Study Type/ Literature Citation	AR Binding	AR Activation	Steroidogenesis	Sex Steroid Hormones	Testes Weight/GSI	Gonad Staging and Histopathology	Epididymides Weight	Epididymides Histopathology	Pituitary Weight	Accessory Sex Organ Weights/ 2° Sex Characteristics	Fertility (Frt)/ Fecundity (Fcd)	Age and Weight at PPS	Vitellogenin	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
Three-Generation Reproduction (Rat; MRID 00120271)					NE	N	NE	N	NE	NE	N			X (H)	X (H)
Chronic Toxicity / Carcinogenicity (Rat; MRID 00069701)					N	N	NE	N	N					X (H)	N
Chronic Toxicity (Dog; MRID 00129600)					N	N	NE	N	N					X (M, H)	X (H)
28-Day Feeding (Rat; MRID 00120267)					↓10% (MH)	NE	NE	NE	N					X (MH,H)	X (H)
Testicular Toxicity (Mouse; Jin *et al*., 2012)				P (H)	P (H)	P (H)								N	N
Testicular Toxicity (Mouse; Wang *et al*., 2012)				N	N	P	N	P						N	N
Avian Reproduction (Quail; MRID 42322901)											N			N	N
Avian Reproduction (Duck; MRID 42322902)											N			N	N[4]
Fish Full Life Cycle (Minnow; MRID 00102096)											N				X (MH, H)

1. Key to responses: L=Low treatment, ML=Medium-low treatment, M=Medium treatment, MH=Medium-high treatment, H=High treatment. Arrows (↓ or ↑) indicate the direction of the response. A shaded cell indicates that is parameter is not routinely evaluated or is not applicable in this assay. Changes in weight are absolute unless otherwise indicated. Abbreviations for androgen sensitive tissues: Seminal vesicles (SV), Ventral prostate (VP), Dorsal prostate (DP), Prostate (PR), Levator ani-bulbocavernosus (LABC), Epididymides (E), Cowper's gland (CG), glans penis (GP).
2. The systemic toxicity in the Tier 1 assays are presented in this column (*e.g*. KW= kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
3. The overt toxicity in the Tier 1 assays are presented in this column (*e.g*. ↓BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
4. Feed consumption at this dose was slightly decreased (NS) throughout the study, with a significant difference from controls during Weeks 6, 12, 14, and 19.

P Positive findings
N Negative findings (*in vitro*)/No effect (*in vivo*)
NE Not examined

C. Effects on Hypothalamic-Pituitary-Thyroidal (HPT) Axis

The current EDSP Tier 1 battery does not have a specific *in vitro* assay to detect chemicals with the potential to affect hypothalamic or pituitary regulation of thyroid hormone production, but it does include three *in vivo* assays that provide data to detect changes in the HPT axis, *i.e*., the pubertal male and female (rat) assays, and the AMA (frog).

The potential interactions of permethrin with thyroid regulation are summarized in **Table 4.** The various targets of the thyroid pathway across the relevant Tier 1 assays are delineated so as to facilitate determination of potential for thyroid or HPT axis effects. This table also includes HPT-relevant findings from data evaluated as OSRI. This table also includes HPG-relevant findings from data evaluated as OSRI. *Effects that occurred in the presence of overt toxicity are discussed in the text but are not reported in the table and not considered further in the WOE assessment.*

In the Tier 1 male pubertal assay, serum T_4 levels were decreased by 28 and 42% at the low (75 mg/kg/day) and high (120 mg/kg/day) doses, respectively, and serum TSH levels were increased by 77% at the high dose. However, the thyroid hormone changes at the high dose occurred in the presence of overt toxicity. No changes were seen in thyroid weight or histopathology. Similarly, in the female pubertal assay, serum T_4 levels were decreased by 26 and 42% at the low (75 mg/kg/day) and high (150 mg/kg/day) doses, respectively, and serum TSH levels were increased by 40% at the high dose. In addition, thyroid weights in females were also decreased by 22% at the high dose, but without a change in thyroid histopathology. It was determined that the high dose in the female pubertal was overtly toxic.

In the AMA, Day-21 normalized hind-limb length (HLL) was increased by 21-23% compared to negative control, however, it was not dose-dependent. Additionally, when Day-21 normalized HLL results were compared to the solvent control, no significant differences were observed. There was no significant acceleration or delay of median Nieuwkoop-Faber (NF) developmental stage at Day 7 or 21 for any treatment level. Further, no asynchronous development was observed. A statistically significant increase in Day 21 wet weight and snout-to-vent length (SVL) was observed at the lowest concentration tested (0.054 µg a.i./L). Finally, effects on thyroid gland histopathology were observed in both controls and all treatment groups. Histopathological effects included thyroid gland atrophy and hypertrophy, follicular cell height increases and decreases, and follicular cell asymmetry. These effects were of low frequency and did not exhibit a concentration-dependent response; therefore, they are not considered to be treatment-related.

In a receptor transactivation study by Du *et al*. (2010), permethrin was observed to have antagonist effects at the thyroid receptor in a transfected African green monkey kidney CV-1 cell line.

In a Part 158 chronic toxicity study in dogs, thyroid weights were increased in a non-dose dependent manner in the low (5 mg/kg/day; ↑27%), mid (100 mg/kg/day; ↑28%) and high (1000 mg/kg/day; ↑17%) dose males, and in the mid (↑27%) and high (↑33%) dose females; additionally, no corroborating histopathological changes were observed.

Table 4. Thyroid Pathway for Permethrin.

Lines of Evidence Indicating Potential Interaction with the Thyroid Pathway for Permethrin[1]										
Study Type/ Literature Citation	TR Binding	Thyroid Weight	Thyroid: Gross and Histopathology	Serum T_4 Levels	Serum TSH levels	Pituitary Weight	Developmental stage (± or asynchronous, HLL)	Growth (BW, SVL)	Systemic Toxicity[2]	Overt Toxicity Observed[3]
EDSP Tier 1 Assay										
Male Pubertal Rat (MRID 48687101)		N	N	↓28% (L)	N	N			↑LW (L, H)	X (H)
Female Pubertal Rat (MRID 48672901)		N	N	↓26% (L)	N	N			↑LW (H)	X (H)
AMA (MRID 48988601)			N				N[4]	Day 21: ↑BW, SVL (L)		N
OSRI										
Receptor Transactivation (Du *et al.*, 2010)	P									
Chronic Toxicity/ Carcinogenicity (Rat; MRID 00069701)			N			N			↑LW; LE (H)	X (H)
28-Day Feeding (Rat; MRID 00120267)			N			N			X (MH, H)	X (H)

1. Key to responses: L=Low treatment, ML=Medium-low treatment, M=Medium treatment, MH=Medium-high treatment, H=High treatment. Arrows (↓ or ↑) indicate the direction of the response. A shaded cell indicates that is parameter is not routinely evaluated or is not applicable in this assay. LW= Liver weight; LE= Liver enzyme
2. The systemic toxicity in the Tier 1 assays are presented in this column (*e.g.* KW= kidney weight). The systemic toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
3. The overt toxicity in the Tier 1 assays are presented in this column (*e.g.* ↓BW). The overt toxicity for the OSRI is indicated by an X in this column. For details see Section IV. A
4. Day-21 normalized hind-limb length (HLL) was increased by 21-23%, however, it was not dose-dependent. Additionally, when Day-21 normalized HLL results were compared to the solvent control, no significant differences were observed

P Positive findings
N Negative findings
NE Not examined

IV. Committee's Assessment of Weight of Evidence

This section of the document describes the weight of evidence (WoE) determination on the potential of cyfluthrin to interact with endocrine related processes (*i.e.,* E, A, or T hormonal pathways) as well as recommendations regarding Tier 2 testing. The results of the Tier 1 assays are considered, along with other scientifically relevant information (*e.g.,* 40 CFR Part 158 test guidelines and published or publicly available peer-reviewed studies). WoE analysis in the context of the EDSP follows the Agency's guidance (USEPA 2011) and is conducted on a case-by-case basis by first assessing the different lines of evidence (*i.e.,* specific Tier 1 assays and OSRI), then performing an integrated analysis of those lines of evidence.

The WoE evaluation includes considerations of biological plausibility of the findings from the different lines of evidence by examining the consistency, coherence and interrelationships among the measured endpoints within and across studies. The available findings from standard toxicology studies on the substance may contribute to the WoE evaluation in helping elucidate if effects seen in the Tier 1 assay are related to perturbations of the endocrine system *per se* or alternatively sequelae of systemic effects. Endocrine modes of action may elicit a number of phenotypic consequences other than those evaluated in the Tier 1 assays.

Endocrine-related findings in the presence of overt toxicity may result in uncertainty as to whether or not the responses are related through an endocrine pathway, therefore non-endocrine toxic responses (including but not limited to mortality or body weight changes) are also considered in this WoE evaluation. The FIFRA SAP that evaluated scientific issues associated with weight of evidence evaluation of the results of the Tier 1 assays stated that *"In summary, the Panel agreed that little, if any, weight should be placed on signs of endocrine disruption in the presence of overt toxicity. All effects in endocrine sensitive tissues should be evaluated in terms of primary interactions with the endocrine system vs. secondary effects related to toxicity in non-endocrine organs or overall disruptions in homeostasis"* (USEPA, 2013).

A. Systemic/Overt Toxicity in the *in vivo* Tier 1 Assays and OSRI

Effects that were considered to be systemic or overt toxicity for the *in vivo* Tier 1 assays and OSRI studies are described below. Generally, one parameter (i.e., death or >10% decrease in mammalian body weight) was sufficient for a dose/concentration to be considered overtly toxic. However, in other instances, more than one parameter was needed to determine overt toxicity. Effects which were considered to be signs of systemic toxicity were generally less severe forms of toxicity (e.g., changes in organ weights or blood chemistry).

1. Tier 1 *in vivo* Assays

Overt and/or systemic toxicity was not observed in the AMA at concentrations up to 0.68 µg a.i./L or in the Hershberger assay at doses up to 120 mg/kg/day. In the FSTRA, overt toxicity was noted at the highest tested concentration (2.6 µg a.i./L) as survival was reduced to 63% for males and 50% for females. Clinical signs of toxicity were also noted in the surviving fish at the high concentration, including erratic swimming, loss of equilibrium and surfacing. Excess feed was also noted in the test chambers on Day 10 and Days 17-19.

In the female pubertal rat assay, systemic toxicity was evident at the high dose (150 mg/kg/day) based the 10% increase ($p<0.05$) in absolute liver weights (both adjusted and unadjusted). Additionally, overt toxicity was noted at the high dose in the female and male pubertal assays. For females, tremors were observed in 4 animals at the low dose (75 mg/kg/d) and in all animals at the high dose. In the male pubertal, three high dose (150 mg/kg/day) rats were found dead after 2 days of dosing; therefore, the high dose was reduced to 120 mg/kg/day. At this reduced dose, one rat was considered uncoordinated on one day. Tremors were observed in 3 animals at the low dose (75 mg/kg/day) and mild to severe tremors in all animals at the high (120 mg/kg/day) at 6 h post-dosing. Additionally, absolute and relative (to body) liver weights were increased ($p<0.05$) by 6 and 11% at the low and high dose, respectively.

2. OSRI

In the three-generation reproduction toxicity study in rats, treatment-related clinical signs in high-dose parental animals were limited to whole body tremors, occurring in all parental generations (exception: tremors were not observed in the P males) during the first few days of the premating period. In the 2500 ppm groups, the incidence rates for the tremors were 20/24 P females, 11/12 and 24/24 F_1 males and females, respectively, and 12/12 and 24/24 F_2 males and females, respectively. Tremors were also observed in pregnant and lactating females exposed to 2500 ppm permethrin. Microscopic examination of F_{3b} weanlings revealed slight to moderate centrilobular hypertrophy, ranging from 0 to 80% for the males and from 10 to 100% for the females.

In a developmental toxicity study in rats, clinical signs of toxicity seen between GD 8-19 included tremors in 21/24 rats and head flicking in 6/24 rats in the high-dose group (150 mg/kg/day). Body weight gains and food consumption by the high-dose dams were less ($p<0.05$) than that of the controls throughout the dosing interval. For GD 7-10, 10-13, and 13-16, body weight gains were decreased by 88%, 32%, and 18%, respectively. Mean fetal body weight of the high-dose group was 3.2% ($p \leq 0.05$) less than that of the controls.

In a developmental toxicity study in rabbits, a total of 0, 5, 5, or 4 does died or were sacrificed moribund in the control, low-, mid- or high-dose groups, respectively. Due to the lack of a dose-response, the deaths could not be definitively attributed to test article administration. Clinical signs

of toxicity included body tremors observed in 5 of the high-dose (1800 ppm) animals. Body weight gains by the low- (600 ppm), mid- (1200 ppm) and high-dose groups were decreased by 79%, 50% and 91%, respectively, during GD 0-18 with significance ($p<0.05$) attained for the low- and high-dose groups. During the post-dosing interval, recovery of body weights was noted for the low- and mid-dose groups, but not for the high-dose group. Post-implantation loss was increased ($p<0.05$) in the mid- and high-dose groups to 155% and 248% of the control level. Correspondingly, the number of early and late resorptions was higher in these groups as compared to the control group values. Mean fetal body weights in the high-dose group were slightly (↓9%; NS) less than that of the controls and attributed to maternal body weight decreases.

In a chronic toxicity/carcinogenicity study in rats, treatment-related tremors and hypersensitivity were observed in the high-dose (2500 ppm) males and females during the first two weeks of the study. Liver changes suggestive of adaptive hypertrophy included increased aminopyrine-*N*-demethylase activity in all male treatment groups in the mid- (1000 ppm) and high-dose female at 52 weeks, and in the high-dose male and female groups at 104 weeks. This was coupled with modestly increased absolute and relative liver weights in the high-dose males and high and low-dose females at 52 weeks and in all male treatment groups and mid-dose females at 104 weeks. Further evidence for adaptive changes included hypertrophy of centrilobular hepatocytes with increased cytoplasmic eosinophilia in the mid- and high-dose male and females at 104 weeks' exposure and increased smooth endoplasmic reticulum proliferation in all treatment groups except low-dose males at 52 weeks and high-dose groups at 104 weeks. Liver changes also included fatty vacuoles confirmed by electron microscopy in the mid- and high-dose males at both 52 and 104 weeks and in the high-dose females at 104 weeks.

In a chronic toxicity study in dogs, neurological clinical signs (tremors, uncoordinated gait, nervousness and convulsions, also excessive salivation and vomiting) were observed in the high-dose group (1000 mg/kg/day). Other findings of systemic toxicity at the high-dose included: decreased body weight gains (↓37% males; ↓33% females), decreased food consumption (increased food left uneaten), increased liver weight (↑30% males; ↑36% females) and alkaline phosphatase levels (↑377% males; ↑220% females). At mid-dose (100 mg/kg/day), increased liver weight (↑25% both sexes) and alkaline phosphatase levels (↑134% males; ↑99% females) were observed. Microscopic evaluation of the adrenals showed focal degeneration and necrosis in the cortex with variable inflammatory cell infiltration along with swelling and vacuolization of the cells in the inner cortex at high-dose males and females (5/5 males and 4/6 females) and at mid-dose males (1/5). The liver also showed hepatic cellular swelling in the mid- and high-dose males and females.

In a 28-day feeding toxicity study in rats, one female and four males died by Day 18 at the high dose (5000 ppm). Surviving rats receiving 2500 ppm and above became hypersensitive during the first week of treatment and remained so throughout the study. Body weight of the 5000 ppm

males was 16-22% below that of controls throughout the treatment period, and overall body weight gain of that group was decreased by 31%. Liver weight was increased 28-33% in the 2500 and 5000 ppm females.

In an avian reproduction toxicity study in quail, systemic toxicity was not observed at the highest dose tested (500 ppm). However, in an avian reproduction toxicity study in ducks, feed consumption was slightly decreased throughout the study, with a significant difference from controls during Weeks 6, 12, 14 and 19 at the high dose (500 ppm).

In a fish full life-cycle toxicity study on fathead minnows, mean measured concentrations of 0.41 ppb significantly reduced the percent survival of fry during the initial 30 days of exposure in the first and second generations. However, minnows which survived the initial exposure period demonstrated normal ranges of measured parameters of survival, growth, reproduction and egg hatchability.

In the published uterotrophic and Hershberger studies in rats by Kim *et al*. (2005), systemic toxicity was not mentioned despite dosing the females at up to 800 mg/kg/day via s.c. injection and the males at up to 100 mg/kg/day via oral gavage. However in a similar study by Kunimatsu *et al*. (2002), tremors were observed in 3-6/6 female rats at the highest dose tested (150 mg/kg/day) and in 2-6/6 male rats at the highest dose tested (75 mg/kg/day). Systemic toxicity was also not observed in the published Hershberger study on rats by Zhang *et al*. (2008) at a single dose level of 50 mg/kg/day (oral dosing). In two other published testicular toxicity studies (Y. Jin *et al*., 2012, and Wang *et al*., 2012), systemic toxicity was not noted in mice dose orally at up to 100 and 35 mg/kg/day, respectively. In a published study by Nillos *et al*. (2010) in medaka, a slight decrease in survival was observed at a concentration of 10 µg/L.

B. Estrogen Pathway

A number of OSRI *in vitro* assays evaluating estrogen receptor activation resulted in conflicting results, *i.e*. some showed activation of estrogen signaling while others did not. The OSRI assays included estrogen receptor (ER) binding, cellular proliferation of estrogen sensitive MCF-7 cells, expression of estrogen sensitive pS2 mRNA in the cancer cells, and interaction with either ERα or ERβ in transfered HeLa cells. Permethrin was negative in the Tier I *in vitro* aromatase assay, whereas in the steroidogenesis assay permethrin caused an increase ($p<0.05$) in estradiol production. *In vivo*, permethrin was negative in both the uterotrophic and female pubertal rat assays, with no effects on uterine weight, mean age at vaginal opening, percent cycling, or histopathological changes in the ovaries and uterus. In the fish short-term reproduction assay (FSTRA), all effects in females were seen only at a dose that caused overt toxicity (i.e, mortality, clinical signs of toxicity). With the exception of one reproductive study in mallard duck which had a slight reduction in the number of eggs laid by the hen (which has limited ability to inform about potential estrogen-related effects by itself and decreases in food consumption also observed at this concentration), no estrogen-related effects were noted in the *in vivo* studies in

mammals, fish, or birds that occurred in the absence of overt and/or systemic toxicity. Therefore, although several of the *in vitro* studies [estrogen receptor (ER) binding, ER transactivation assay (ERTA), and steroidogenesis] indicated that permethrin had the potential to affect the E pathway at the cellular level, these effects are not supported by the available *in vivo* studies.

C. Androgen Pathway

For AR binding, conflicting results were reported across the *in vitro* assays with different cell lines. In an *in vitro* assay, permethrin reduced sperm motility in a concentration-dependent manner (1-64 µmol/L). Multiple Hershberger assays were available for permethrin, in which the Tier 1 assays as well as an additional open literature study (Kunimatsu, *et al*., 2002) were negative at doses up to 75 or 120 mg/kg/day. However, in another OSRI Hershberger assay (Kim *et al*., 2005), significant decreases in AST weights were reported at doses of ≥10 mg/kg/day.

In the male rat pubertal assay, no androgen-related effects were observed. While there does not appear to be androgen-related effects in rats with an intact HPG axis (i.e. male pubertal and Part 158 rat data); published results in the literature (Jin *et al*., 2012, Wang *et al*., 2012) reported that permethrin caused testicular toxicity in mice following oral exposure at a dose of 35 or 100 mg/kg/day. In these studies, testicular toxicity manifested as changes in testosterone levels, decreased testicular and epididymal weights, changes to the epididymal duct, regression of sperm quality and histopathological lesions in the testes. In the FSTRA, all effects in males were only observed at the high concentration in the presence of overt toxicity.

Therefore based on the available *in vitro* and mammalian *in vivo* data, there appears to be a potential interaction with the androgen pathway in mammals. There was no convincing evidence of a potential interaction with the androgen pathway in wildlife.

D. Thyroid Pathway

There was no convincing evidence of an interaction of permethrin with the thyroid pathway in mammals or wildlife in the absence of overt toxicity. The only thyroid-related effect observed in the absence of overt toxicity was a decrease in serum T4 levels in the male and female pubertal assays. No thyroid related effects were observed in the mammalian Part 158 studies. No treatment-related thyroid effects were seen in the AMA.

E. Conclusions

There is no convincing evidence of potential interaction with the estrogen or thyroid pathways in mammals or wildlife. There is evidence for potential interaction with the androgen pathway in mammals. There is no convincing evidence for a potential interaction with the androgen pathway in wildlife.

V. EDSP Tier 2 Testing Recommendations

For permethrin, a point of departure (POD) of 25 mg/kg/day for human health risk assessment is based on neurotoxic clinical signs (abnormal and/or decreased movement, increased body temperature, and aggression) observed at 75 mg/kg/day (LOAEL) in an acute neurotoxicity study in rats. This POD is used for deriving the chronic Reference Dose (RfD) for chronic dietary risk assessment. A second POD of 11 mg/kg/day based on clinical signs of toxicity (body temperature and hypersensitivity to noise) seen at 154 mg/kg/day (LOAEL) in a subchronic inhalation toxicity study in rats. This POD is used for calculating the Margins of Exposure (MOEs) for non-dietary risk assessment. These PODs are lower than the dose (100 mg/kg/day) that caused the testicular effects in mice. Consequently, the current RfD and MOEs are protective of the androgen-mediate effects observed in the published literature study. Additionally, there was no evidence of interaction with the androgen pathway in the EDSP Tier 1 assays. Therefore, there is no concerns for the findings reported in the literature study.

Based on weight of evidence considerations, mammalian EDSP Tier 2 testing is not recommended for permethrin since additional testing is not expected to impact EPA's current regulatory point of departures and endpoints for human health risk assessments.

Given the lack of evidence for a potential interaction with the estrogen and androgen pathway in fish and thyroid pathway in amphibians, no additional testing is recommended.

For birds, Part 158 avian reproduction studies with both northern bobwhite quail and mallard duck are available for permethrin. In the bobwhite quail, no reproductive effects were observed, but effects on reproductive parameters were observed in the mallard duck. The type of data obtained from Part 158 avian reproduction studies (OCSPP 850.2300) are considered sufficient for evaluating potential reproductive effects to birds from permethrin exposure. Relative to the EDSP, additional testing is not recommended.

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APPENDIX 1. EDSP Tier 1 Screening Assays

Amphibian Metamorphosis Assay; (AMA, OCSPP 890.1100)

The 21-day assay (MRID 48988601) of permethrin (97.7% purity) on amphibian metamorphosis of African clawed frogs (*Xenopus laevis*) was conducted under flow-through conditions. Amphibian larvae at developmental Nieuwkoop-Faber Stage (NF) 51 (80; 20/replicate) were exposed to control (water only; negative), a solvent control (0.020 mL/L dimethylformamide; DMF), and permethrin at nominal concentrations of 0.00011, 0.00033, and 0.0010 mg a.i./L; mean-measured concentrations were <0.0000600 (<LOQ; controls), 0.000052, 0.00022, and 0.00063 mg a.i./L. The reviewer-calculated time-weighted average concentrations were <0.0000600 (<LOQ; controls), 0.000054, 0.00022, and 0.00068 mg a.i./L. The test system was maintained at 21.5 to 23.0°C and a pH of 7.9 to 8.2.

Unless otherwise indicated, all effects are reported based on comparison to the negative (clean water) control.
Two non-incidental mortalities were observed in the test, one each in the low and high treatment groups. Clinical signs included tail curvature, which was observed in both controls and all treatment groups (12-28% of individuals), with no indication of a dose-dependent pattern.

Compared to the negative control, permethrin significantly promoted Day 21 normalized hind-limb length (HLL) at the low and mid treatment groups (by 21% and 23% respectively). There were no effects on normalized HLL on Day 7. However, there was an apparent solvent effect (21% stimulation of normalized HLL, p=0.01) relative to the negative control at Day 21. When Day 21 normalized HLL results were compared to the solvent control, no significant differences were observed. There was no significant acceleration or delay of median NF developmental stage at Day 7 or 21 for any treatment level. Further, no asynchronous development was observed. Compared to the negative control, there was a statistically significant increase in Day 21 wet weight and snout-to-vent length (SVL) at the low treatment group. No solvent effect was seen for SVL.

Thyroid gland histopathology observations included thyroid gland atrophy and hypertrophy, follicular cell height increases and decreases, and/or follicular cell asymmetry in both controls and all treatment groups. These effects were of low frequency and did not exhibit a concentration-dependent response; therefore, they are not considered to be treatment-related.

Androgen Receptor (AR) Binding Assay; (OCSPP 890.1150)

Requirement satisfied by OSRI (Xu *et al.,* 2008; Zhang *et al.,* 2008a; Tyler *et al.*, 2000; Zhang *et al.,* 2008b; Kim *et al.,* 2005); see Appendix 2.

Aromatase Assay; (OCSPP 890.1200)

In an *in vitro* aromatase (CYP 19) assay (MRID 48618601), permethrin (97.7% a.i., Lot # PMPS000014) was incubated with human recombinant aromatase and tritiated androstenedione (1-β [^{3}H(N)]-androst-4-ene-3,17-dione; [^{3}H]ASDN) in dimethyl sulfoxide (DMSO) at log concentrations of 10^{-10} to 10^{-3} M for 15 minutes to assess the potential of permethrin to inhibit aromatase activity.

Aromatase activity was determined by measuring the amount of tritiated water produced at the end of a 15-minute incubation for each concentration of chemical. Tritiated water was quantified using liquid scintillation counting (LSC). Three runs were conducted and each run included a full activity control, a background activity control, a positive control series (10^{-10} to 10^{-5} M) using a known inhibitor (4-hydroxyandrostenedione; 4-OH ASDN), and the permethrin series (10^{-10} to 10^{-3} M) with three repetitions per concentration.

Aromatase activity in the full activity controls was 0.588 ± 0.079 nmol·mg-protein^{-1}·min^{-1}. The response of each full activity control within a run was between 90 to 110% of the average full activity, and the activity in the background controls ranged from 0.21 to 0.31% and averaged 0.28% of the full activity. For the positive control substance (4-OH ASDN), aromatase activity results were within the recommended ranges for the performance criteria. The estimated log IC_{50} for 4-OH ASDN averaged −7.3 M and the Hill slope was −1.0.

Precipitation was noted at the two highest concentrations of permethrin tested (10^{-4} and 10^{-3} M); therefore, the maximum concentration used for data analysis was $10^{-4.5}$ M. The lowest portion of the response curve was greater than 75% activity at all soluble concentrations of permethrin. Aromatase activity averaged 101.3% and 101.9% activity at concentrations of 10^{-10} and $10^{-4.5}$ M permethrin, respectively, indicating that permethrin did not inhibit aromatase activity in this assay.

Based on the data from the average response curve, permethrin is classified as a Non-inhibitor of aromatase activity in this assay.

Estrogen Receptor (ER) Binding Assay; (OCSPP 890.1250)

Requirement satisfied by OSRI (Saito *et al*., 2000); see Appendix 2.

ERα Transcriptional Activation (ERTA) Assay; (OCSPP 890.1300)

Requirement satisfied by OSRI (Tyler *et al*., 2000); see Appendix 2.

Fish Short Term Reproduction Assay; (FSTRA, OCSPP 890.1350)

The 21-day short-term reproduction assay (MRID 48702301) of permethrin with fathead minnow (*Pimephales promelas*) was conducted under flow-through conditions. Adult fish (6 months old, 2 males and 4 females per replicate) were exposed to permethrin (97.7% purity) at nominal concentrations of 0 (negative and solvent controls), 0.00033, 0.00099, and 0.0030 mg a.i./L. Time-weighted average (TWA) concentrations were <0.000150 (<LOQ; controls), 0.00022, 0.00075, and 0.0026 mg a.i./L. The solvent control consisted of dimethylformamide (DMF) at a final concentration of 0.02 mL/L. The test system was maintained at 24.6 to 25.8°C and a pH of 7.9 to 8.1.

Unless otherwise indicated, all effects are reported based on comparison to the negative (clean water) control. Survival in the negative control and the low and mid treatment groups was 100%, but there was 63% survival for males (5 of 8) and 50% survival for females (8 of 16) at the high treatment concentration. Additionally, clinical signs of toxicity occurring in the high treatment group included erratic swimming (5 fish), loss of equilibrium (2 fish), and surfacing (2 fish). There were also observations of excess feed noted in the test chambers of the high treatment group on Day 10 and Days 17-19. These observations indicate that overt toxicity was present during the course of this study at the high treatment concentration.

Fecundity in the negative control averaged 19.8 eggs/female/reproductive day with all replicates having at least 15 eggs/female/reproductive day; spawning occurred at least every 4 days. Fertilization success in the negative control averaged 95.4%. Permethrin had no significant effect on fecundity or fertility in any treatment group relative to the negative control ($p>0.05$). While not statistically significant ($p>0.05$), there was a 42% decrease on fecundity at the high treatment concentration.

Plasma vitellogenin (VTG) concentrations were comparable between the negative control and treatment groups for both sexes. While not statistically significant ($p>0.05$), there was a 37.8% reduction in VTG as compared to the negative control at the high treatment concentration. The data from this treatment group were based on three replicates and not four (as the rest of the treatment groups) as 3 of 4 female fish from Replicate A died during the course of the study.

Effects on gonadal histopathology were observed in female fish at the high treatment level, including increased post-atretic follicles, decreased post-ovulatory follicles, and granulomatous inflammation. Microsporidia were observed in one fish each of the control and treatment concentrations that showed a minimal to mild severity of granulomatous inflammation. There were no effects of treatment on gonadal histopathology in male fish.

A significant increase ($p<0.05$) in gonado-somatic index (GSI) was detected at the high treatment level for both sexes (↑48.9% for males and 28.4% for females). Nuptial tubercle score was significantly decreased in males at the high concentration (<0.05). Permethrin exposure was not associated with any significant changes ($p>0.05$) in body weight or length for either sex.

Other clinical signs (*i.e.*, behavioral and other sublethal effects) including bloody lower jaw and weakness were observed in 1 to 3 of the 8 surviving females at the high treatment level.

Hershberger Assay; (OCSPP 890.1400)

In a Hershberger assay (MRID 48618602) screening for androgenic activity, permethrin (97.7% a.i., Lot# PMPS000014) in corn oil was administered daily via oral gavage to groups of 59 to 60-day old, castrated male Sprague Dawley rats (8/group) at dose levels of 0 (vehicle), 37.5, or 120 mg/kg/day. An androgenic positive control group consisting of eight castrated rats exposed to 0.4 mg/kg/day testosterone propionate (TP) by subcutaneous (s.c.) injection was also included as a positive control.

To screen for potential anti-androgenic activity, permethrin in corn oil was also administered daily via oral gavage to 59 to 60-day old, castrated male Sprague Dawley rats (8/group) at dose levels of 12, 37.5, 120 mg/kg/day in conjunction with a daily dose of reference androgen TP at 0.4 mg/kg/day by s.c. injection. The control group consisted of eight castrated male rats dosed daily with TP (0.4 mg/kg/day) by s.c. injection in corn oil, and the anti-androgenic positive control group consisted of eight castrated male rats dosed orally with flutamide (FT) in corn oil at 3 mg/kg/day in conjunction with a daily s.c. dose of TP (0.4 mg/kg/day).

For both components of the assay, the animals were dosed for 10 consecutive days and necropsied approximately 24 hours after the final dose administration to determine weights of the five androgen-dependent tissues.

All animals survived until scheduled termination. No clinical signs of toxicity were observed in the permethrin dose groups of the androgen agonist or antagonist assays. Body weights and overall body weight gains in the treated groups were similar to controls in both the androgen agonist and anti-androgen assays.

In the androgen agonist assay, animals dosed with TP had increased ($p<0.05$) body weights (↑12%) on Day 11, and increased ($p<0.05$) overall body weight gains (↑110%) compared to controls. No significant increases in the organ weights were observed in any of the five accessory sex tissues of animals dosed with permethrin. Animals in the TP group had increases ($p\leq0.05$) in accessory sex organ weights as follows: 1002% in seminal vesicles; 898% in ventral prostate; 187% in levator ani-bulbocavernosus (LABC); 486% in Cowper's gland; and 51% in

glans penis. For the vehicle control, the maximum guideline-specified %CV was exceeded for the LABC (45% vs. 30%). All other %CV values were less than the maximum permissible values.

In the anti-androgen assay positive control group (TP + FT), overall body weight gains were decreased ($p \leq 0.05$) by 35%. No significant decreases in the organ weights were observed in any of the five accessory sex tissues of animals dosed with permethrin+TP. Animals dosed with TP + FT (positive control) had decreased ($p \leq 0.05$) accessory sex organ weights as follows: 84% in seminal vesicles; 77% in ventral prostate; 60% in LABC; 62% in Cowper's gland; and 20% in glans penis. The maximum guideline-specified %CV was exceeded for the Cowper's gland in the 120 mg/kg/day permethrin group (22% vs. 20%). All other %CV values were less than the maximum permissible values.

The dosing for this study (high-dose of 120 mg/kg/day) was considered adequate based on data from two earlier studies showing either mortality at doses ≥200 mg/kg/day or clinical signs of neurotoxicity at 150 mg/kg/day.

Statistically significant changes were not seen in two or more of the five androgen sensitive tissue weights. Permethrin was negative for androgenicity and anti-androgenicity in the Hershberger assay.

Female Pubertal Assay; (OCSPP 890.1450)

In a Female Pubertal Assay (MRID48672901), 16 Sprague-Dawley (Crl:CD® [SD] IGS) female rats/dose group were treated daily via oral gavage with permethrin (Lot # PMPS00014, 97.7% a.i.) at doses of 0 (corn oil) 75, or 150 mg/kg/day from post-natal day (PND) 22 to 42/43. Animals were examined for vaginal opening (VO) daily beginning on PND 22, and age and weight at attainment of VO were recorded. Following sacrifice on PND 42/43, blood was collected for clinical chemistry analyses, including analysis of total thyroxine (T_4) and thyroid stimulating hormone (TSH) using a radioimmunoassay (RIA). Adrenal, liver, thyroid, pituitary and urogenital organ weights were recorded, and microscopic examinations were performed on the ovaries, uterus, thyroid, and kidneys.

All rats administered permethrin survived until schedule termination. From PND 34 onward, tremors were observed in 4 animals administered 75 mg/kg/day and all animals administered 150 mg/kg/day at 4-6 hours post-dosing on at least one day. These findings did not persist to the daily examinations, which were conducted just prior to dosing.

No treatment-related effects were observed on body weights, body weight gains, age of attainment of VO, or weight at day of attainment at any dose. All surviving animals attained complete VO.

Absolute liver weights (adjusted and unadjusted) were increased ($p<0.05$) by 10% at 150 mg/kg/day. Relative (to body weight) liver weights were increased ($p<0.05$) by 4 and 8% at 75 and 150 mg/kg/day. Both unadjusted and adjusted thyroid weights were decreased ($p<0.05$) by 22% at 150 mg/kg/day.

Administration of permethrin did not change the day of first estrus compared to the vehicle control group. Animals exhibited similar cycle lengths and regularity. The majority of females in the 150 mg/kg/day group were in diestrus at necropsy.

Serum T_4 levels were decreased ($p<0.05$) by 26 and 42% at 75 and 150 mg/kg/day, respectively, and, serum TSH levels were increased ($p<0.05$) by 40% at 150 mg/kg/day. Additionally, at 150 mg/kg/day, increases ($p<0.05$) were observed in sodium (↑3%), chloride (↑4%), and sorbitol dehydrogenase (SDH; ↑20%), alkaline phosphatase (ALP) was decreased (↓16%).

There were no effects of treatment in the thyroid on either colloid area or follicular cell height in any treated group. No effects of treatment were observed microscopically in the ovaries, uterus or kidney.

The dose levels tested were determined to be adequate since they were based on the results of the range finding study where mortality was seen at doses greater than 200 mg/kg/day and tremors at 150 mg/kg/day.

Male Pubertal Assay; (OCSPP 890.1500)

In a Male Pubertal Assay (MRID 48687101), 16 Sprague-Dawley [Crl:CD(SD) IGS] rats/dose group were treated daily via oral gavage with permethrin (97.7% a.i., Lot # PMPS00014) in corn oil at doses of 0, 75, or 150 mg/kg/day from postnatal day (PND) 23 to 53/54. However, three 150 mg/kg/day rats were found dead after two days of dosing. Therefore, the high dose was reduced to 120 mg/kg/day on PND 25. Animals were examined for preputial separation (PPS) daily beginning on PND 30, and age and weight on day of attainment were recorded. Following sacrifice on PND 53/54, blood was collected for clinical chemistry and hormone analyses. Total serum testosterone, thyroxine (T_4) and thyroid stimulating hormone (TSH) levels were determined using radioimmunoassays. Adrenal, liver, pituitary, thyroid, and urogenital organs were weighed and examined macroscopically. The left testes, left epididymides, left kidney, and thyroid were examined microscopically.

No treatment-related related effects were observed on body weights, body weight gains, age or weight at attainment of PPS, organ weights (except liver), clinical chemistry, and gross or histopathology parameters.

After reduction of the high dose to 120 mg/kg/day, all remaining animals survived to study termination. At 6 hours post-dose, tremors were observed at 75 and 120 mg/kg/day in a dose-dependent manner, with incidence, frequency, and severity increasing with dose. At 75 mg/kg/day, three animals exhibited mild tremors on one day and one animal exhibited mild tremors on two days. At 120 mg/kg/day, very mild to severe tremors were observed in all surviving animals on several days. Additionally, one 120 mg/kg/day rat was considered uncoordinated on one day.

Serum testosterone levels in the treated groups were similar to controls. Mean serum T_4 levels were decreased ($p<0.001$) by 28 and 42% at 75 and 120 mg/kg/day, respectively, and serum TSH level was increased ($p<0.05$) by 77% at 120 mg/kg/day. The T_4 and TSH values were all within or just below their respective reference ranges. Additionally, there were no concomitant effects observed on thyroid weights or thyroid follicular cell height or colloid area. Absolute and relative liver weight were increased ($p<0.05$) by 6-11% at 75 and 120 mg/kg/day. There were no changes in clinical chemistry parameters that suggested hepatotoxicity due to permethrin administration. The increased liver weights were considered to be the result of hypertrophy and associated microsomal enzyme induction, a common finding with permethrin.

The 150 mg/kg/day dose was an excessive dose level as three rats were found dead on Day 2. Therefore, the dose was lowered to 120 mg/kg/day which was an adequate high dose based on the clinical signs (tremor and in coordination) seen in these animals. Tremors were also noted at 75 mg/kg/day.

Steroidogenesis Assay; (OCSPP 890.1550)

In a steroidogenesis assay (MRID 48618603) H295R cells cultured *in vitro* in 24-well plates were incubated with permethrin (97.7% a.i.; Batch PMPS000014) at concentrations of 0.0001, 0.001, 0.01, 0.1, 1, 10 and 100 µM in triplicate for 48 hours. Dimethyl sulfoxide (DMSO) was used as the vehicle, at a final concentration of 0.05%.

Testosterone and estradiol levels were measured using HPLC/MS-MS. Three independent experiments were performed (Run #s 2-4). A Quality Control (QC) plate was run concurrently with each independent run of a test chemical plate to demonstrate that the assay responded properly to positive control agents at two concentration levels; positive controls included the known inhibitor (prochloraz) and inducer (forskolin) of estradiol and testosterone production.

Guideline acceptability recommendations and requirements were met, including lack of cytotoxicity, adequate production of testosterone and estradiol, acceptable reproducibility (low %CV) and appropriate induction and inhibition with positive controls.

No cytotoxicity was observed over the range of tested permethrin concentrations. However, precipitation of the permethrin was observed at the 100 μM concentration in each run and at 10 μM in Run #2. Accordingly, the data from these concentrations were not included in the analysis of the respective runs. For permethrin, there was a statistically significant ($p<0.05$) increase in testosterone production at 10 μM in Runs #3 and #4 (average 1.14-fold), and a statistically significant increase in estradiol production at 1 and 10 μM in Runs #3 and #4 (average 1.16-fold for 1 μM and 1.51-fold for 10 μM).

Based on hormone responses in two of the three independent runs, permethrin treatment resulted in statistically significant and reproducible increases in testosterone and estradiol production.

Uterotrophic Assay; (OCSPP 890.1600)

Requirement satisfied by OSRI (Kim *et al*., 2005; Kunimatsu *et al*., 2002); see Appendix 2.

APPENDIX 2. Other Scientifically Relevant Information (OSRI)

ER Transactivation and Binding Assays

In a published study by **Saito *et al.*** (**MRID 48176003**), the estrogenic and anti-estrogenic activity of permethrin (>93% purity) was evaluated using three *in vitro* assays based on hERα-related mechanisms: 1) a mammalian cell-based luciferase reporter assay, 2) a yeast two-hybrid method, and 3) a competitive ligand-binding assay. For the luciferase reporter assay, HeLa cells were transiently transfected with an expression vector, a reporter plasmid for *h*ERα, and a luciferase control plasmid. Permethrin was added to the medium at concentrations of 100 nM to 10 μM. For anti-estrogenic activity measurements, 100 pM of E_2 was incubated along with permethrin. For the yeast two-hybrid assay, yeast transformants were grown overnight and then treated with permethrin at 10 μM (maximum solubility). For antagonist assays, 100 pM of E_2 was incubated with permethrin. After incubation, induced β-galactosidase was measured by chemiluminescent detection. Competitive binding to *h*ERα was assayed by a fluorescence polarization method. Test chemical-dependent displacement of a labeling ligand, fluoromone ES1, from ERα was measured by changes in fluorescence anisotropy. Dose-dependent displacement of fluormone ES1 from *h*ERα was detected with the positive controls DES, *p*-nonylphenol, and HTM.

In the luciferase reporter assay, permethrin was tested at a maximum dose of 30 μM and no statistically significant estrogenic or anti-estrogenic activity was detected. No statistically significant estrogenic or antiestrogenic activity for permethrin was detected in the yeast two-hybrid assay or the receptor binding assay. The lack of statistically significant effects at concentrations of 100 nM-10 μM indicates that permethrin is not estrogenic or anti-estrogenic by classic ER-related pathways *in vitro*.

Recombinant Yeast Assay

In a published study by **Tyler et al. (MRID 48213601)**, permethrin was tested for estrogen and androgen receptor binding activity (both agonist and antagonist) using a recombinant yeast assay. Two yeast strains were stably transfected to express either the human ER (hER) or the AR (hAR) genes along with the *lacZ* reporter gene. In addition to permethrin, three major derivatives of permethrin resulting from metabolism and/or environmental degradation were similarly tested: cyclopropane permethrin acid, 3-PBOH, and 3-phenoxybenzoic acid (3-PBA). Permethrin from four separate chemical suppliers was tested (purities 97.3-99.4%). In screens for agonistic activity, E_2 and 5α-dihydrotestosterone (DHT) were used as positive controls. The positive controls in the anti-estrogen and anti-androgen screens were hydroxytamoxifen and flutamide, respectively. EC_{50}s and IC_{50}s were calculated relative to the reference standards, and for all chemicals inducing a response, LOEC (agonist) and/or LOIC (antagonists) were determined relative to the reference standards.

Permethrin was a very weak estrogen agonist with potencies between seven and eight orders of magnitude less than E_2. In the anti-estrogen screen, increasing concentrations of permethrin produced a progressive increase in the E_2-stimulated background absorbance, indicating an additive estrogenic response. In the hAR assay, very high concentrations of permethrin (~10^{-3} M) induced slight increases in activity above baseline values, thus indicating very weak agonist activity. Permethrin also exhibited weak anti-androgenic activity with a potency approximately 500-fold less than flutamide. The following activities were presented for permethrin: Estrogenic LOEC = $3.1 \pm 0.4 \times 10^{-4}$ M; estrogenic $EC_{50} = 2 \times 10^{-3}$ M; anti-androgenic LOIC = $3.0 \pm 0.57 \times 10^{-5}$ M; anti-androgenic $IC_{50} = 7.3 \pm 1.4 \times 10^{-4}$ M.

All three of the permethrin metabolites had endocrine activity. 3-Phenoxybenzyl alcohol had both estrogenic and anti-androgenic activity and was approximately 10^{-5}-fold less potent than E_2, but only 4-fold less potent than flutamide. 3-Phenoxybenzoic acid and permethrin cyclopropane acid both had anti-estrogenic activity and were approximately 100-fold and 1,000-fold less potent than 4-OH-tamoxifen, respectively. In the anti-estrogen screen, an increasing concentration of 3-PBOH produced progressive enhancement of the E_2-stimulated absorbance, showing an additive estrogenic response. At concentrations greater than 8×10^{-3} M, 3-PBOH was toxic to the yeast and caused lysis of the cells.

Uterotrophic Assay (Rat)

In a multi-component study, **Kim *et al.* (MRID 48176002, 2005)** examined the estrogenic effects of permethrin (96.6% purity) using Northern blot analysis of uterine Calbindin-D_{9k} gene expression (CaBP-9k) and an uterotrophic assay using immature (18-day-old) female SD rats. CaBP-9k is an intracellular calcium binding protein that is estrogen-responsive in the rat uterus. Uterine tissues obtained during the uterotrophic assay were used for the Northern blot analysis. Permethrin (5, 10, 50, 100, 200, or 800 mg/kg) alone, plus E_2 (3 µg/kg), or plus ICI 182,780 (3 µg/kg) was administered to immature female rats by s.c. injection. For the uterotrophic assay, animals were sacrificed 24 h after the 3-day treatment, and the uterus and vagina were removed and weighed. For the CaBP-9k assay, total RNA from uterine tissues was extracted for Northern blot analysis of CaBP-9k mRNA. A description of the probe used was not provided.

High dose (800 mg/kg) permethrin increased ($p<0.05$) relative (to body) uterine weights (data presented graphically). Permethrin also enhanced E_2-induced uterine weight increases (data presented graphically); however, this was maximal at 200 mg/kg ($p<0.05$). It was stated that the permethrin-induced increases in relative uterine weights were inhibited by co-treatment with ICI 182,780; however, the inhibitory effect was statistically different only at 50 mg/kg ($p<0.01$), and control weights were not provided. Relative vaginal weight was unaffected by treatment with permethrin. Northern blot analysis showed an up-regulation in CaBP-9k mRNA following permethrin exposure that was maximal at the lowest dose (5 mg/kg). Permethrin also enhanced the E_2-induced increase in CaBP-9k mRNA levels in a non-dose-dependent fashion.

Uterotrophic (Rat)

In a multi-component study, **Kunimatsu *et al.* (MRID 48176008, 2002)** performed a uterotrophic assay with permethrin (97.4% purity) using 5-week-old ovariectomized female Sprague-Dawley [Crj:CD(SD)IGS] rats. Groups of six females were dosed with permethrin in corn oil via oral gavage at dose levels of 0, 37.5, 75, or 150 mg/kg/day for 3 days. Two additional groups received ethynyl estradiol (30 µg/kg/day) or methoxychlor (125 mg/kg/day) as reference chemicals. One day after the final dose, rats were euthanized and uterus, liver, and kidneys were dissected and weighed. For the uterus, both wet (with luminal contents) and blotted (without luminal contents) weights were determined.

Tremor was observed in 3-6/6 rats in the high-dose (150 mg/kg/day) group. No increases in uterus weight (wet or blotted) were observed. Reference controls ethynyl estradiol and methoxychlor both showed the expected effects. The results of the study provide no evidence that permethrin exhibits any potential to cause adverse (anti-) estrogenic effects at dose levels below those causing excessive systemic toxicity.

Receptor Transactivation Assays

In a published study by **Du *et al.* 2010** (**MRID 48196301**), permethrin and its metabolite 3-PBA were tested for ER, AR, and thyroid hormone receptor (TR) binding activity. Agonist and antagonist interactions with the ER and TR were examined using transfected African green monkey kidney CV-1 cells and AR activation was determined using transfected MDA-kb2 cells. The agonist assays were conducted using permethrin (94.0% purity) at concentrations ranging from 10^{-9} M to 10^{-5} M; to test antagonist activity, the ER, AR, and TR tests were conducted at the same concentrations of permethrin in the presence of E_2, DHT, and T_3, respectively. The REC_{20} (relative effective concentration, agonist) and RIC_{20} (relative inhibitory concentration, antagonist) were calculated.

Permethrin exhibited weak estrogen agonistic effects at concentrations from 10^{-8} to 10^{-5} M ($REC_{20} = 8.10\times10^{-7}$ M) and also exhibited weak antagonist effects at 10^{-6} and 10^{-5} M, in the presence of 1×10^{-9} M E_2 ($RIC_{20} > 10^{-5}$ M). The metabolite 3-PBA showed antagonist effects at 10^{-6} and 10^{-5} M, in the presence of 1×10^{-9} M E_2 ($RIC_{20} = 8.84\times10^{-8}$ M). No AR agonist activity was found for permethrin or 3-PBA; however, permethrin and 3-PBA showed AR antagonist activity with a calculated RIC_{20} of 1.88 x 10^{-6} M for permethrin and $>10^{-5}$ M for 3-PBA. Similarly, permethrin and 3-PBA did not exhibit TR agonist activity but did show TR antagonist activity with a calculated RIC_{20} of 1.50×10^{-9} M for permethrin and 4.76×10^{-6} M for 3-PBA.

ER Transactivation and Binding Assays

In a published study by **Chen *et al.*** (**MRID 48160705**), the effect of permethrin (purity ≥90%) on cell proliferation in human breast cancer estrogen-sensitive (MCF-7) cells in the absence of

estrogens (negative control) and in the presence of 17β-estradiol (E_2, positive control) was investigated (E-Screen assay). A binding assay using rat uterine cytosol ER was also studied and estrogen-responsive pS2 mRNA expression in MCF-7 cells treated with permethrin was measured by RT-PCR.

For the cell proliferation assay, MCF-7 cells were plated and permethrin was tested in log increments at concentrations from 10^{-11} to 10^{-6} M. The bioassay was terminated on Day 6 and proliferation was determined by absorbance measurements after addition of thiazolyl blue solution. The proliferative effect (PE) is measured as the ratio between the highest cell yield obtained with the test chemical and the hormone free control and is expressed as -fold proliferation. Estrogenic activity was assessed by relative proliferative potency (RPP) which is the ratio of maximum PE for the chemical to PE for 17β-estradiol (E_2).

For the ER competitive-binding assay, 10^{-9} M [^{3}H]-estradiol was incubated with increasing concentrations of permethrin, uterine cytosol preparation, and buffer. The bound ligand was separated from the free ligand and radioactivity was measured. Data for permethrin and the E_2 standard curve were plotted as percent [^{3}H]-E_2 bound vs. molar concentration, and the IC_{50} was determined. Permethrin was tested in log increments at concentrations from 10^{-12} to 10^{-3} M.

For the pS2 mRNA assay, MCF-7 cells in medium were treated with permethrin or E_2 at various concentrations. All cells were harvested at the same time, total RNA was extracted, and mRNA for pS2 and β-actin were amplified by RT-PCR.

In the E-Screen assay, an inverse U-shaped dose response curve was obtained that was significant at 10^{-10} to 10^{-7} M, with a maximum proliferation response at 10^{-8} M. The PE value for permethrin was 2.69. In comparison with the PE of E_2 (3.46), permethrin showed a partial agonistic response. In the ER competitive-binding assay, permethrin inhibited the binding of [^{3}H]-E_2 at concentrations greater than 10^{-7} M, at a maximum of <67%. The pS2 mRNA expression increased 1.28-fold after 10^{-7} M permethrin treatment.

ER Transactivation and Binding Assays

In a published study by **Kim *et al.*** (**MRID 48176007**), the estrogenic activity of permethrin was evaluated using the E-screen assay (with the MCF-7 cell line) and a competitive ER binding assay. Cells were incubated for six days in the presence of permethrin (98% purity; concentrations not reported) prior to being assayed for cell proliferation. The competitive ER binding assay was conducted using uterine cytosols isolated from Sprague-Dawley rats. Aliquots of rat uterus cytosol were incubated with selected concentrations of permethrin and [^{3}H]-estradiol.

In the E-screen assay, the positive control, E_2, induced cell proliferation in a dose dependent manner. Permethrin did not induce MCF-7B BUS cell proliferation at any of the concentrations

tested. To evaluate potential anti-estrogenic activity, cells were treated for six days at a concentration of 10^{-6} M permethrin in the presence of either 10^{-10} or 10^{-11} M E_2. 10^{-6} M permethrin significantly inhibited E_2-induced cell proliferation. In the competitive ER binding assay, permethrin did not compete with [^{3}H]-estradiol at any of the concentrations tested**.**

ER Transactivation Assays

In a published study by **M. Jin *et al.*** (**2010**), permethrin (99% purity) and its metabolites PBCOH (98% purity), PBCHO (≥97% purity), and 3-PBA (98% purity) were tested for estrogenic activities in the MCF-7 cell line. A cell proliferation assay was conducted using MCF-7 cells incubated with medium containing 10^{-9} M E_2 (positive control), the test chemicals at 0.001-10 μM, or ethanol (0.1%, v/v; negative control). After 5 days, cell proliferation was determined by measuring the absorbance with a micro plate reader. The proliferative effect (PE), the ratio of the maximal cell yield of the test compound to the cell yield of the negative control, and the relative proliferative effect (RPE), the ratio of the PE for the test chemical to the PE of E_2, were calculated for assessment of the estrogenicity of the chemicals.

Estrogenic activity was also evaluated using a gene expression assay. MCF-7 cells were incubated for two days with the test chemicals at 0.001-10 μM, E_2 at 10^{-9} M (positive control), or ethanol at 0.1% (v/v; negative control). Total RNA was extracted and reverse transcribed for quantitative PCR analyses for the genes pS2 (an estrogen-responsive protein in breast tumors) and ERα. Based on the results of preliminary experiments, quantitative PCR measurements were conducted with the test chemicals at 10^{-6} M.

Permethrin induced proliferation ($p<0.05$) of MCF-7 cells at 10^{-8} to 10^{-6} M compared to the solvent control; RPE values at 10^{-7} M were determined to be 56.3% and 55.4%, compared to 10^{-9} M E_2 (100%). The three metabolites induced cell proliferation to various degrees with RPE values for PBCOH, PBCHO, and 3-PBA determined as 62.5%, 15.2%, and 28.6%, respectively. Permethrin and the metabolites PBCOH and 3-PBA induced proliferation of MCF-7 cells in a concentration-related manner; it was noted that the nonlinear response seemed to follow an inverted U-shaped dose-response pattern with the most significant effects observed at a concentration of 10^{-7} M.

All test chemicals were found to up-regulate pS2 transcription. Permethrin and the metabolite PBCOH induced pS2 expression 2.59- and 3.45-fold, respectively, compared to the control. The effects of the metabolites PBCHO and 3-PBA on the pS2 expression were much lower (1.37- and 1.85-fold, respectively) than the other test chemicals. Permethrin and PBCOH at 10^{-6} M decreased the ERα mRNA expression to levels similar to E_2, which was found to down-regulate ERα by 50% compared to the control. Metabolites PBCHO and 3-PBA down-regulated the ERα expression levels at lower levels (3.7% and 12.0%, respectively). The relative inductive efficiency of the test chemicals on the up-regulation of pS2 expression compared to that of E_2 and the relative inhibitory efficiency of the test chemicals on the down-regulation of ERα

regulation compared to that of E_2 were calculated. In the pS2 expression assay, PBCOH showed a fully estrogenic response (82%), and permethrin and 3-PBA showed a partially estrogenic response (29-53%); PBCHO showed only a weakly estrogenic response (13%). Similarly, in the ERα expression assay, PBCOH a showed fully estrogenic response (89%), and permethrin showed a partially estrogenic response (69%); 3-PBA showed only a weakly estrogenic response (24%), and the PBCHO response was <10% (negative response).

Estrogenic Potential

In a published study by **Garey and Wolff (MRID 48176005, 1998)**, permethrin (95.7% purity) was evaluated for estrogen and progesterone agonist/antagonist activities using two *in vitro* systems based on human cells (Ishikawa Variant-I human endometrial cancer cell line for estrogen effects and T47D human breast cancer cell line for progesterone effects). Both cell lines produce alkaline phosphatase (ALP) as an indicator of hormonal activity. Test compounds and standards were dissolved in ethanol and diluted in medium, added to cells in plates, and incubated. After a 48-h exposure period, cells were rinsed and a *p*-nitrophenyl phosphate solution was added to each well. The resulting formation of *p*-nitrophenol was detected using a plate reader.

In Ishikawa Var-I cells, permethrin did not demonstrate estrogen agonist or antagonist activities; in T47D cells, permethrin did not exhibit progesterone agonist or antagonist activities.

Estrogenic Potential

In a published study by **Go *et al.* (MRID 48176004)**, permethrin (95.7% purity) was evaluated for estrogenic potential in the MCF-7 estrogen-sensitive cell line, as measured by expression of pS2 and cell proliferation. For the pS2 expression assay, MCF-7 cells were incubated for 3 days and then exposed to permethrin for 48 hours. Total RNA was extracted and pS2 RNA was detected by Northern blot and autoradiography with quantification by densitometry. Estradiol (E_2) was used as the positive control. Results were expressed as the ratio of permethrin to the vehicle control. In the MCF-7 cell proliferation assay, cells were exposed to permethrin at concentrations of 0.001-100 µM. Cell proliferation was determined on Day 6 by counting with a Coulter counter.

Permethrin did not induce pS2 above the negative control or inhibit E_2 induction of pS2 in initial experiments at 5 µM, but at higher concentrations (100 µM), it weakly induced pS2 expression. In combination with 0.1 nM E_2, permethrin had little effect on pS2 expression levels stimulated by E_2. At concentrations below 10 µM, permethrin did not induce MCF-7 cell growth, but induced modest increases in proliferation at 100 µM.

ER Transcriptional Activation Assay

In a published study by **Lemaire *et al.* (MRID 48074114)**, permethrin was evaluated for ERα and ERβ activation or inhibition in stable reporter cell lines. Stable transfection of the ERα and ERβ constructs together with an estrogen reporter luciferase vector into HeLa cells resulted in two E_2-sensitive cell lines. The cells were incubated with permethrin (≥95% purity) for 16 h. At the end of incubation, a commercial luciferase assay system was added and luciferase activity was measured with a luminometer. For the ERα and ERβ competitive-binding assay, ERα and ERβ cells were treated with 0.1 nM [^{3}H]-E_2 in the presence or absence of increasing concentrations of unlabeled E_2 (as control) or permethrin. Total cell lysate was counted by liquid scintillation.

With the transactivation activity of E_2 set at 100%, the estrogenic activity percentages of 10 µM permethrin were 13.0 ± 0.4% for ERα and 14.2 ± 2.4% for ERβ; these were not statistically different from the control values of 9.3 ± 1.3% and 10.8 ± 1.6%, respectively. Permethrin showed no anti-estrogenic activity in either ERα or ERβ.

AR Binding Assay

In a published study by **Bauer *et al.*** (**MRID 48176001, 2002**), permethrin (97.5% purity) was evaluated for its AR binding capability using an immuno-immobilized androgen receptor assay (IRA) with recombinant human androgen receptor (rhAR). The receptor preparation was diluted and incubated with ^{3}H-DHT in the presence or absence of increasing concentrations of permethrin or DHT. After washing unbound radioactivity, wells were separated and transferred into scintillation vials and radioactivity was counted. Specific binding was calculated as the difference of total binding and non-specific binding in the presence of a 200-fold surplus of unlabelled DHT. The relative binding affinity of permethrin was compared to DHT using the inhibition constants (K_i) of each compound to calculate the relative binding affinity, where RBA = (K_i(DHT) × 100) ÷ K_i(permethrin).

The K_i (permethrin) could not be determined because there was no detectable displacement of DHT up to the highest concentration tested (unreported). Therefore the permethrin RBA was reported as <0.01%.

Androgen Receptor Transactivation Assays

In a published study by **Xu *et al.*** (**MRID 48176006**), the potential androgenic and anti-androgenic activities of permethrin (>98% purity) were evaluated using a transiently-transfected human AR reporter gene assay in an African monkey kidney cell line (CV-1). After a 24 h incubation period, 0.1% ethanol (vehicle control) or 1 nM DHT (positive control), and various concentrations of permethrin (10^{-7} to 10^{-5} M) were added for measurement of agonistic or antagonistic activity. The cells were harvested 24 h after dosing, lysed, and the lysate analyzed

for chloramphenicol transferase (CAT) and β-galactosidase (β-Gal) activities using commercial kits. The amount of CAT for each lysate was normalized to the β-Gal activity.

There were no indications of cytotoxicity at the dose levels tested; there was no significant difference in the amounts of β-Gal between the treated groups and vehicle control groups. Permethrin did not exhibit androgenic activity at the tested concentrations. Permethrin did attenuate an increase in the CAT amount induced by DHT; the inhibitory effect was detected at 1×10^{-5} M. The IC_{50} value was $(5.86 \pm 2.20) \times 10^{-5}$ M.

Androgen Receptor Transactivation Assays

As part of a multi-component published study by **Zhang *et al.* (MRID 48196302, 2008)**, the potential anti-androgenic effects of permethrin (purity not reported) were examined using an androgen-responsive cell line (MDA-kb2). Various concentrations of permethrin with and without DHT (1 nM) were tested.

Permethrin was found to significantly inhibit DHT-induced transcriptional activation at a concentration of 10^{-5} M (data presented graphically).

Hershberger Assay (Rat)

In a multi-component study, **Kim *et al.* (MRID 48176002)** conducted a Hershberger assay with permethrin (96.6% purity) using 5-week-old male SD rats. Male SD rats were castrated at 5 weeks of age. Permethrin was administered by oral gavage at 10, 50 or 100 mg/kg/day for 10 days (n=6–8). As a reference androgen, 0.4 mg/kg TP was injected subcutaneously. To examine anti-androgenic activity in a second group of rats, TP-treated rats (n=6–8) were administered permethrin at 10, 50 or 100 mg/kg/day for 10 days. Flutamide (25 mg/kg/day) and *p,p´*-DDE (100 mg/kg/day) were used as reference anti-androgens. Animals were evaluated daily for mortality, injury and general appearance, and individual body weights were recorded every three days. Twenty-four hours after the final dose the five androgen-dependent tissues were excised and weighed.

No statistically significant changes in body weights were noted in any of the treatment groups. The positive control, TP, induced statistically significant increases in the absolute and relative weights of all of the androgen-dependent sex accessory tissues. Permethrin did not show an agonist effect on androgen-dependent tissue weights at any dose tested. Flutamide and *p,p´*-DDE reduced sex accessory tissue weights in TP-treated castrated males verses the TP-treated group. Administration of permethrin to TP-treated castrated rats led to statistically significant reductions (antagonism) in the five sex accessory tissue weights compared to TP at all doses tested, though not in a dose-dependent manner, nor to the extent of flutamide or *p,p´*-DDE.

Hershberger Assay (Rat)

In a multi-component study, **Kunimatsu *et al.* (MRID 48176008)** conducted a 5-day Hershberger assay with permethrin (97.4% purity) using 9-week-old castrated male Sprague-Dawley [Crj:CD(SD)IGS] rats. Groups of six males were dosed with permethrin in corn oil via oral gavage at dose levels of 0, 25, 50, or 75 mg/kg/day for 5 days with or without 0.25 mg/kg/day TP administered by s.c. injection as a reference androgen (eight groups). For the 4 groups treated with TP, an additional group received 100 mg/kg/day *p,p´*-DDE (gavage) as a reference control for assessing weak anti-androgenic activity. For the groups receiving no TP, an additional group received 100 mg/kg/day methyltestosterone via gavage as a reference control for androgenicity. One day after the final administration of permethrin, rats were euthanized, the sex accessory glands/tissues (ventral prostate, dorsolateral prostate, seminal vesicles with coagulating glands and LABC), liver and kidneys were dissected and weighed.

Tremor was noted in 2-6/6 rats at 75 mg/kg/day. No toxicologically significant changes were observed in body weight, food consumption, or organ weights. No androgenic or anti-androgenic effects resulted from treatment with permethrin. Reference controls *p,p´*-DDE and methyltestosterone produced the expected effects. The results of the study provide no evidence that permethrin exhibits any potential to cause adverse (anti-) androgenic effects at dose levels below those causing excessive systemic toxicity.

Hershberger Assay (Rat)

As part of a multi-component published study by **Zhang *et al.* (MRID 48196302, 2008)**, a Hershberger assay was conducted with permethrin (purity not reported) using 3-week-old male Sprague-Dawley rats. After 7 days acclimation, the rats were castrated and allowed to recover for 2 weeks. Following recovery, rats were weighed and randomly assigned to experimental groups (n=6). The groups consisted of controls (0.5 mg/kg/day TP s.c.), TP + flutamide (50 mg/kg/day p.o.), and TP + permethrin (50 mg/kg/day orally). The treatment period was 7 days for all groups. Following the last dose, rats were euthanized by exsanguination. The seminal vesicles, ventral prostate, dorsolateral prostate, LABC muscle, Cowper's glands, glans penis, liver, paired adrenal glands, spleen and paired kidneys were dissected and weighed. Serum testosterone was determined using commercially available FIA kits.

No mortality or treatment-related clinical signs were noted in any of the groups. It was reported that there were no significant differences between initial or final body weights, or weight gains of the groups compared to the control group. Permethrin caused significant decreases in seminal vesicle and ventral prostate weight (data presented graphically).

Three-Generation Reproduction Toxicity (Rat, OCSPP 870.3800)

In a two-generation reproduction toxicity study (**MRID 00120271**), permethrin (94.0-98.8% purity) was administered to groups of 12 male and 24 female Wistar rats in the diet at concentrations of 0, 500, 1000, or 2500 ppm (0, 25, 50, and 125 mg/kg/day, respectively, using a standard conversion factor of 0.05). Two litters were produced by each generation. Parental animals in each generation received test or control diet for 12 weeks post-weaning and were then paired for mating to produce the A litters. After various rest periods, the parental animals were mated again to produce the B litters. Test diets were administered during mating, gestation and lactation for three successive generations throughout the study. The F_2 parents were mated for a third time, using the same breeding pairs as for the B litters, producing the C litters for a developmental toxicity evaluation. Ten males of the F_1 generation were maintained on experimental diets until they were 54-55 weeks old and were submitted for microscopic examination of selected neurological tissues.

There were no treatment-related parental deaths. A few parental animals were euthanized for humane reasons. There were no treatment-related effects on body weights, body weight gains, food consumption, or food efficiency.

Treatment-related clinical signs in high-dose parental animals were limited to whole body tremors, occurring in all parental generations (exception: tremors were not observed in the P males) during the first few days of the premating period. In the 2500 ppm groups, the incidence rates for the tremors were 20/24 (P females), 11/12 and 24/24 (F_1 males and females, respectively), and 12/12 and 24/24 (F_2 males and females, respectively). Tremors were also observed in pregnant and lactating females exposed to 2500 ppm permethrin. The tremors were intermittent and transient. Neuropathy was not observed in a special microscopic examination of selected neurological tissues from F_1 males continued on test for one year.

Gross examination at necropsy did not reveal any dose- or treatment-related findings, nor did microscopic examination of grossly abnormal tissues from all parents surviving to scheduled termination and of reproductive tissues from animals suspected of infertility.

The LOAEL for systemic toxicity is 2500 ppm (125 mg/kg/day) based on tremors observed in the P females, and the F_1 and F_2 males and females. The systemic toxicity NOAEL is 1000 ppm (50 mg/kg/day).

There were no treatment-related effects in the reproductive performances (male or female fertility indices, gestation index, gestation length, live birth index, viability index, lactation index, parturition, or fetal sex ratio) of the F_1, F_2 and F_3 generations. In the F_{3c} litters, there were no developmental effects associated with the administration of permethrin over three generations. The percentages of male fetuses of the 1000 and 2500 ppm groups (39.0 and 44.7%, respectively) were lower than the control value (53.2%), but the effect was not associated

with increased resorptions and was not dose-related. Also, no consistent effect on sex ratios was observed in other litters or generations of the study.

Microscopic examination of F_{3b} weanlings revealed dose-related increases in centrilobular hypertrophy of the liver. The incidences of slight and moderate centrilobular hypertrophy were dose-related, ranging from 0 to 80% for the males and from 10 to 100% for the females. The hypertrophy of the liver was an adaptive and reversible effect and was not considered adverse.

The reproductive toxicity and offspring toxicity NOAEL is ≥2500 ppm (125 mg/kg/day) and the reproductive toxicity and offspring toxicity LOAEL is not identified.

Developmental Toxicity (Rat, OCSPP 870.3700)

In a developmental toxicity study (**MRID 40943603**), 24 presumed pregnant Wistar rats per group were administered 0, 15, 50, or 150 mg/kg/day of permethrin (93.9% purity; 38 cis:62 trans isomers) in corn oil by oral gavage on gestation days (GD) 7-16, inclusive. On GD 22, all surviving dams were sacrificed and all fetuses were weighed, sexed, and examined for external, visceral, and skeletal malformations/variations.

All dams survived to scheduled termination and no treatment-related abnormalities were noted at necropsy. There were no effects of treatment on clinical signs, body weight gains, or food consumption in the low- or mid-dose groups. In the high-dose group, clinical signs of toxicity seen between GD 8-19 included tremors in 21/24 rats and head flicking in 6/24 rats. Body weight gains and food consumption by the high-dose dams were less ($p<0.05$) than that of the controls throughout the dosing interval. For GD 7-10, 10-13, and 13-16, body weight gains were decreased by 88%, 32%, and 18%, respectively.

The maternal LOAEL is 150 mg/kg/day based on clinical signs of toxicity and decreased body weight gain and food consumption. The maternal NOAEL is 50 mg/kg/day.

No dose- or treatment-related effects were observed on gravid uterine weights, fetal sex ratios, pre- or post-implantation losses, or numbers of corpora lutea/dam or live fetuses/dam. Mean fetal body weight of the high-dose group was 3.2% ($p \leq 0.05$) less than that of the controls. However, mean litter weight of the high-dose group was 3% (NS) greater than that of the controls. Therefore, the reduced fetal body weights were considered a questionable toxic response.

No treatment-related external or visceral fetal malformations/variations were noted. The fetal and litter incidence rates of short length extra ribs were increased ($p<0.05$) in the high-dose group as compared with the controls. Short length extra ribs were observed in 31% of the high-dose fetuses vs. 11% of the control fetuses and in 87% of high-dose litters vs. 57% of control litters.

The developmental LOAEL is 150 mg/kg/day based on decrease in fetal body weights and an increase in the incidence rate of short length extra ribs. The developmental NOAEL is 50 mg/kg/day.

Developmental Toxicity (Rabbit, OCSPP 870.3700)

In a prenatal developmental toxicity study (**MRID 40943602**), presumed pregnant Dutch rabbits were administered 0, 600, 1200, or 1800 mg/kg/day of permethrin (92.5% purity; 32.3 cis:60.2 trans isomers) in 0.5% aqueous Tween 80 by oral gavage on GD 6-18, inclusive. The number of does mated for each group was 19, 21, 20, and 23, respectively. On GD 29, all surviving does were sacrificed and all fetuses were weighed and examined for external malformations/variations. Approximately one-half of the fetuses were processed for skeletal examination and the remaining one-half was fixed and examined for visceral anomalies.

A total of 0, 5, 5, or 4 does died or were sacrificed moribund in the control, low-, mid-, or high-dose groups, respectively. Due to the lack of a dose-response, the deaths could not be definitively attributed to test article administration. Clinical signs of toxicity included body tremors observed in 5 of the high-dose animals only. Little or no feces or urine was noted on at least one occasion for 2/19 (11%), 4/21 (19%), 6/20 (30%), and 8/23 (35%) animals in the control, low-, mid-, and high-dose groups, respectively.

Absolute body weights were similar between the treated and control groups throughout the study. However, after examining the replotted body weight data, there was a sharp drop in weight for the low, mid, and high dose groups after Day 6 and only a slight drop for the control that was noticeable after Day 12. Body weight gains by the low-, mid-, and high-dose groups were decreased by 79%, 50%, and 91%, respectively, during GD 0-18 with significance ($p<0.05$) attained for the low- and high-dose groups. During the post-dosing interval, recovery of body weights was noted for the low- and mid-dose groups, but not for the high-dose group.

The maternal LOAEL is estimated to be <600 mg/kg/day based on decreased body weight gain. The maternal NOAEL is not identified.

The number of live fetuses and mean litter size was decreased for all dose groups compared to the control group (110(15), 80(13), 69(14), and 72(13) for control, low-, mid-, and high-dose groups, respectively). However, no dose-response was evident or statistical significance noted.

Post-implantation loss was increased ($p<0.05$) in the mid- and high-dose groups to 155% and 248% of the control level. Correspondingly, the number of early and late resorptions was higher in these groups as compared to the control group values. Mean fetal body weights in the high-dose group were slightly (↓9%; NS) less than that of the controls and attributed to maternal body weight decreases. No dose-related or statistical differences were observed between the treated and control groups for number of fetuses/litter or mean gravid uterine weights.

No treatment-related external or visceral fetal malformations/variations were noted. In the mid- and high-dose groups, reduced ossification of the fore- and hindlimbs was indicated by slightly (NS) greater ossification scores as compared with the controls. Mean scores for the control, low-, mid-, and high-dose groups were 1.92, 1.99, 2.00, and 2.25, respectively, for the forelimb and 1.65, 1.56, 1.89, and 1.90, respectively, for the hindlimb.

Therefore, the developmental LOAEL is 1200 mg/kg/day based on increased post-implantation loss, greater numbers of early and late resorptions and an equivocal decrease in ossification of the fore- and hindlimbs. The developmental NOAEL is 600 mg/kg/day.

Chronic Toxicity/Carcinogenicity (Rat, OCSPP 870.4300)

In a combined chronic toxicity/carcinogenicity study (**MRID 00069701**), permethrin was administered to 60 rats/sex/group in the feed at doses of 0, 500, 1000, or 2500 ppm (mean estimated intake 0/0, 19.4/19.1, 36.9/40.2, and 91.5/104 mg/kg/day in males/females, respectively). Twelve rats/sex/group were sacrificed at 52 weeks and the surviving rats were sacrificed at 104 weeks exposure.

There was no treatment-related effect on mortality or tumor induction. During the first two weeks of the study, treatment-related tremors and hypersensitivity were observed in the high-dose males and females. There were no effects on body weight, body weight gain, food consumption or food efficiency, ophthalmologic endpoints, hematologic endpoints, clinical chemistry, or urinalysis parameters.

Liver changes suggestive of adaptive hypertrophy included increased aminopyrine-*N*-demethylase activity in all male treatment groups, in the mid- and high-dose female at 52 weeks, and in the high-dose male and female groups at 104 weeks. This was coupled with modestly increased absolute and relative liver weights in the high-dose males and high and low-dose females at 52 weeks and in all male treatment groups and mid-dose females at 104 weeks. Further evidence for adaptive changes included hypertrophy of centrilobular hepatocytes with increased cytoplasmic eosinophilia in the mid- and high-dose male and females at 104 weeks' exposure and increased smooth endoplasmic reticulum proliferation in all treatment groups except low-dose males at 52 weeks and high-dose groups at 104 weeks. Liver changes also included fatty vacuoles confirmed by electron microscopy in the mid- and high-dose males at both 52 and 104 weeks and in the high-dose females at 104 weeks. It was concluded that the increased liver weight and hypertrophy observed in the liver were adaptive and reversible and were not adverse.

Under the conditions of this study, the LOAEL is 2500 ppm (91.5/104 mg/kg/day in males/females, respectively) based on tremors and hypersensitivity. The NOAEL is 1000 ppm (36.9/40.2 mg/kg/day in males/females, respectively).

At the doses tested, permethrin did not affect the incidence of tumor-bearing animals or the incidence of any specific tumor type in either sex. Permethrin was not carcinogenic to the rat. Dosing was considered adequate based on liver effects and on tremors and hypersensitivity in male and female rats.

No treatment-related changes were seen in absolute or relative weights of the testes, ovaries, pituitary, or adrenal glands at any dose level. No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicle, prostate, ovaries, uterus, cervix, mammary, thyroid, adrenal or pituitary glands at any dose level.

Chronic Toxicity (Dog, OCSPP 870.4100)

In a chronic toxicity study (**MRID 00129600**), permethrin (92.5%, purity, cis/trans 32.3/60.2) was administered to beagle dogs (6/sex/group) in corn oil by gelatin capsule at dose levels of 0, 5, 100, or 1000 mg/kg/day for one year. The high dose was lowered from 2000 mg/kg/day after 2 days due to overt toxic reaction to the test material.

There were no mortalities. Neurological clinical signs (tremors, uncoordinated gait, nervousness and convulsions, also excessive salivation and vomiting) were observed in the high-dose group. At the high-dose, decreased body weight gain (37% for males and 33% for females less than control, respectively), decreased food consumption (increased food left uneaten), increased liver weight (+30% and +36% for males and females, respectively) and alkaline phosphatase level (+377% and +220% for males and females, respectively) were reported. At mid-dose, increased liver weight (+25% both sexes) and alkaline phosphatase levels (+134% for males and +99% for females) were observed.

The thyroid showed apparent increases in weight for the males (+27%, +28% and +17%) and the females (-5%, +27% and +33%) for the low, mid and high dose test groups. No treatment-related changes were seen in absolute or relative weights of the testes, ovaries, adrenal or pituitary glands at any dose level. No treatment-related histopathological lesions were seen in the testes, epididymides, prostate, ovaries, uterus, cervix, mammary, thyroid, or pituitary glands at any dose level. Microscopic evaluation of the adrenals showed focal degeneration and necrosis in the cortex with variable inflammatory cell infiltration along with swelling and vacuolization of the cells in the inner cortex at high-dose males and females (5/5 males and 4/6 females) and at mid-dose males (1/5). The liver also showed hepatic cellular swelling at mid- and high-dose males and females.

The observations of increased liver weight, alkaline phosphatase levels, and hepatic cellular swelling were considered adaptive and reversible and were not adverse. Therefore, the systemic toxicity LOAEL is 1000 mg/kg/day based on clinical neurotoxic signs and decreased body weight gain and food consumption. The NOAEL is 100 mg/kg/day.

28-Day Oral (Feeding) Toxicity (Rat)

In a 28-day oral toxicity range-finding study (**MRID 00120267**), permethrin (90.5% purity) was administered to Wistar rats (8/sex/dose) in the diet at dose levels of 0, 200, 500, 1000, 2500, 5000, or 10,000 ppm (equivalent to 0/0, 20/20, 51/51, 106/76, 267/207, and 319/428 mg/kg/day in males/females, respectively). The mg/kg equivalent of the 10,000 ppm dose could not be calculated, since all rats receiving that dose died within three days.

At 5000 ppm, one female and four males died by Day 18. No other deaths were observed. Surviving rats receiving 2500 ppm and above became hypersensitive during the first week of treatment and remained so throughout the study. Body weight of the 5000 ppm males was 16-22% below that of controls throughout the treatment period, and overall body weight gain of that group was decreased by 31%. Liver weight was increased 28-33% in the 2500 and 5000 ppm females. The relative (to body) liver weight was increased in the 2500 and 5000 ppm males, and in all treated females except those receiving 500 ppm. The liver changes were judged to be an adaptive response. Testis weight was decreased 10-13% in 2500- and 5000-ppm males, and relative adrenals weight was decreased 29% in 5000-ppm females; neither of these organ weight changes showed a dose-response relationship. There were no treatment-related changes in food consumption, hematology, clinical chemistry, or urinalysis of any group of rats. No treatment-related changes were seen in absolute or relative weights of the ovaries or pituitary glands at any dose level. No treatment-related histopathological lesions were seen in the testes, epididymides, seminal vesicle, prostate, ovaries, uterus, mammary, thyroid, adrenal or pituitary glands at any dose level.

Under the conditions of this study, the LOAEL for permethrin in rats is 2500 ppm (equivalent to 267/207 mg/kg/day for males/females, respectively) based on hypersensitivity. The NOAEL is 1000 ppm (equivalent to 106/76 mg/kg/day for males/females, respectively).

Testicular Toxicity (Mouse)

In a published study by **Y. Jin *et al.*** (**2012**), 5 male ICR mice/dose group were administered one of the four permethrin enantiomers, (+)-cis-, (−)-cis-, (+)-trans-, and (−)-trans-permethrin (separated from racemic permethrin, >95% purity), in DMSO orally at dose levels of 0 (DMSO), 25, 50, or 100 mg/kg/day from PND 21 to PND 42. Mice were sacrificed on the last day of treatment. Blood was collected, and serum testosterone levels were measured using a radioimmunoassay. The testes were weighed, and the left testis in each group was fixed, processed, sectioned, and stained with hematoxylin and eosin for histological analysis. Total RNA was isolated from the testes of mice and then reverse transcribed for quantitative PCR to determine the expression of 3-hydroxy-3-methylglutaryl-CoA synthase (HMG-CoA synthase), 3-hydroxy-3-methylglutaryl-CoA reductase (HMC-CoA reductase), low-density lipoprotein receptor (LDL-R), scavenger receptor class B type 1 (SR-B1, high-density lipoprotein receptor), peripheral benzodiazepine receptor (PBR), steroidogenic acute regulatory protein (StAR),

cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), cytochrome P450 17α-hydroxysteroid dehydrogenase (P450 17α), and 17β-hydroxysteroid dehydrogenase (17β-HSD).

No clinical signs of toxicity were observed and there were no effects of treatment on body weight. At 100 mg/kg, both absolute and relative weights of testes in the (+)-cis, (−)-cis and (−)-trans-permethrin treated groups were decreased significantly ($p<0.05$) compared to the control; no significant differences were found in the (+)-trans-permethrin treated group. No histological changes in the testes were observed in mice exposed to 25 or 50 mg/kg of any of the permethrin enantiomers. However, the number of spermatogenic cells was decreased in the (+)-cis, (−)-cis, and (−)-trans-permethrin treated groups at 100 mg/kg. Large interstitial spaces in the seminiferous tubules were also observed in the 100 mg/kg (+)-cis, (−)-cis, and (−)-trans-permethrin treated groups. At 100 mg/kg (+)-cis, (−)-cis, and (−)-trans permethrin exposure, serum testosterone levels were significantly reduced compared to the control. However, when the groups treated with the different enantiomer types were compared to each other, no significant differences in testosterone concentration were observed at any dose.

The effects of permethrin enantiomer exposure on the mRNA levels of the main genes related to cholesterol synthesis in the testes were evaluated, as cholesterol is the main precursor for steroid hormones such as testosterone. The mRNA levels of HMG-CoA synthase decreased significantly in the testes of the mice after treatment with 25, 50, and 100 mg/kg (+)-cis, (−)-cis, (+)-trans, and (−)-trans enantiomers compared to the control; however, no significant differences were observed between the 4 enantiomers. For HMG-CoA reductase, significant differences in mRNA levels were not observed between the 25 mg/kg enantiomer-treated mice and control or between the different enantiomer groups. At 50 and 100 mg/kg, enantiomer-specific responses were observed. The mRNA levels of HMG-CoA reductase were only significantly decreased compared to the control in the 50 and 100 mg/kg (+)-cis enantiomer treated groups (↓40% and 48%, respectively). Significant differences were also found between the (+)-cis and (−)-cis or (−)-trans groups at 50 mg/kg dosage. The mRNA levels of testicular SR-B1 in all permethrin enantiomer treated groups were slightly inhibited, but did not attain statistical significance. No significant differences in LDL-R mRNA levels were observed in any permethrin enantiomer-treated group compared to the control

PBR and StAR play key regulatory roles in cholesterol transport from the outer to the inner mitochondrial membrane. PBR mRNA levels were decreased by permethrin treatment in dose-dependent and enantiomer-specific manners. At 25 and 50 mg/kg exposure levels, the testicular mRNA level of PBR was decreased significantly only in the (+)-cis enantiomer treatment group, while at 100 mg/kg it was significantly decreased in all enantiomer groups compared with the control group (↓39%, 47%, 47%, and 33%, respectively). An enantiomer-specific effect on PBR expression was observed at both 25 and 50 mg/kg concentrations; a significant difference in PBR mRNA expression was found between the (+)-cis and (−)-trans groups. The testicular StAR

mRNA levels were down-regulated significantly in the 25, 50, and 100 mg/kg (+)-cis and (−)-trans enantiomer-treated groups compared to control. Additionally, StAR mRNA levels were down-regulated in the (−)-cis and (+)-trans enantiomer-treated groups at 50 mg/kg and the (−)-cis enantiomer group at 100 mg/kg. Moreover, significant differences were also found between the (+)-trans and (−)-trans groups at 100 mg/kg.

Key genes in the testosterone synthetic pathway, including P450scc, 3β-SHD, P450 17α and 17β-HSD, in the testes of mice were also examined. A significant decrease in P450scc mRNA levels appeared in the 25 mg/kg (+)-cis and (−)-trans enantiomer groups compared to controls. At 50 or 100 mg/kg, the testicular P450scc mRNA levels were significantly inhibited by all four enantiomers. Treatment with the (+)-cis enantiomer induced a dose-dependent decrease in 3β-HSD mRNA levels, with significance reached at 50 and 100 mg/kg (+)-cis enantiomer (↓30% and 46%, respectively), and treatment with the (-)-cis enantiomer resulted in a significant decrease at 100 mg/kg. The mRNA levels of P450 17α were not influenced significantly regardless of the permethrin enantiomers or administration doses. Significant differences in the mRNA levels of testicular P450scc, 3β-HSD and P450 17α were not observed between the permethrin enantiomers for any dose level. However, the effects on 17β-HSD appeared in a different manner. First, a significant decrease was observed in the (+)-cis enantiomer in all groups (25, 50, and 100 mg/kg) compared to the control. Second, at 100 mg/kg, gene expression was decreased significantly for all the permethrin enantiomers. Third, there was a significant difference between the (+)-cis and (+)-trans groups and between the (-)-cis and (+)-trans groups at 25 and 50 mg/kg.

Testicular Toxicity (Mouse)

In a published study by **Wang *et al.*** (**2012**), nine-week-old male Sv/129 mice were dosed by daily oral gavage with 10 µmol/kg diazinon (equivalent to 3 mg/kg), 90 µmol/kg cis-permethrin (99.5%; equivalent to 35 mg/kg), or diazinon+cis-permethrin at 100 µmol/kg for 6 weeks; a control group was dosed with corn oil alone. After the first treatment and just before the last treatment, mice were placed in individual cages to collect urine for 24 hours; levels of cis-permethrin and metabolite 3-PBA were determined in urine by GC/MS. Mice were terminated 16 hours after the final treatment. Blood was collected and plasma cis-permethrin (GC/MS) and testosterone (enzyme immunoassay) levels were measured. Liver, epididymis, testis, seminal vesicle, and prostate were collected and weighed. The levels of cis-permethrin in testis were measured by GC/MS. The mRNA levels of StAR (steroidogenic acute regulatory protein), TSPO (translocator protein), and P450scc (cytochrome P450 side-chain cleavage) genes in testes were measured by real-time quantitative PCR. Epididymal sperm suspension was used to microscopically assess sperm count, sperm motility, and abnormalities in sperm morphology. Western blot analyses were conducted on testis homogenate with primary antibodies against StAR, TSPO, and P450scc. Histopathological evaluation of the right testes and caput epididymides was conducted after the tissues were fixed in modified Davidson's solution,

processed, and stained with periodic acid Schiff's reagent then hematoxylin. Seminiferous tubules were classified into three stage groups (I-VI, VII-VIII, and IX-XII) and examined for changes of tubule atrophy, cytoplasm vacuolation, and germ cell degeneration.

No signs of systemic toxicity were observed during dosing, and there were no effects of treatment on body weights, body weight gains, or reproductive organ weights. Urinary excretion of 3-PBA was decreased ($p<0.05$) in mice in the mixture group compared to the cis-permethrin group (↓30-50%). Levels of cis-permethrin in plasma and testes were 1.5- and 1.8-fold higher, respectively, in mice in the mixture group compared to the cis-permethrin group. These results indicate that metabolism of cis-permethrin is inhibited by diazinon. Plasma testosterone levels in the diazinon and cis-permethrin treatment groups were similar to controls; however, plasma testosterone levels were decreased ($p<0.01$) in the mixture group compared to controls (↓45%) and compared to the cis-permethrin group (↓32%). Testicular mRNA and protein expression of StAR were not significantly different in any of the treatment groups compared to the controls. TSPO mRNA levels and protein expressions were significantly decreased in the cis-permethrin (↓46% and 47%, respectively) and mixture (↓53% and 55%, respectively) groups compared to controls. There were no significant differences between the cis-permethrin and mixture groups for TSPO mRNA and protein expressions. For P450scc, mRNA levels were significantly decreased (↓70%) in the mixture group compared to controls; P450scc mRNA levels in the cis-permethrin groups were similar to controls. Protein expression of P450scc was significantly decreased in all treatment groups compared to controls (↓24% and 43% for the cis-permethrin and mixture groups, respectively). Two-way ANOVA did not show any significant interaction between exposure and testosterone levels or mRNA or protein expressions of StAR, TSPO, or P450scc.

Histopathological examination of the testes showed no significant increase in abnormalities in the cis-permethrin groups compared to controls; however, the numbers of degenerate germ cells in seminiferous tubules was significantly increased in the mixture group compared to controls. The percentage of seminiferous tubules in stage group VII-VIII was significantly decreased in the cis-permethrin and mixture groups. In the caput epididymis, cytoplasmic vacuolation was observed more frequently in all treatment groups than in the controls; median vacuolation scores were significantly higher in the treatment groups. Two-way ANOVA did not show any significant interaction between exposure to both chemicals and testicular or epididymal changes. Caudal epididymal sperm count was significantly reduced in the cis-permethrin (↓60%) and mixture (↓51%) groups compared to controls. Sperm motility was also significantly decreased in the cis-permethrin and mixture groups. The percentage of sperm with normal morphology was significantly decreased in the mixture (↓17%) group compared to controls; the decrease in normal sperm morphology in the mixture group was also significant when compared to the cis-permethrin group. Two-way ANOVA did not show any significant interaction between exposure to both chemicals and sperm count, percent motility, or percent abnormal morphology.

Avian Reproduction Toxicity (Quail, OCSPP 850.2300)

In an avian reproduction toxicity study (**MRID 42322901, 1992**), groups of 32 bobwhite quail (*Colinus virginianus*) were fed diets containing permethrin at 0, 25, 125, or 500 ppm. The birds were fed the diets beginning at 20 weeks of age and continuing through egg laying, final incubation, hatching, and a 14-day offspring rearing period; the total approximate duration of treatment was 27 weeks. The birds were housed with one male and one female per pen, with 16 pens per dose group.

Adult quail showed no overt signs of toxicity and no treatment-related clinical signs. There were no treatment-related mortalities. There were three deaths in the 25 ppm group which were considered to be incidental to treatment as no treatment-related findings were seen at necropsy.

There were no significant differences in body weights, feed consumption, reproductive parameters, egg shell thickness, or offspring body weight between the control and any treatment group.

Avian Reproduction Toxicity (Duck, OCSPP 850.2300)

In an avian reproduction toxicity study (**MRID 42322902**), groups of 32 Mallard ducks (*Anas platyrhyncos*) were fed diets containing permethrin at 0, 25, 125, or 500 ppm. The birds were fed the diets beginning at 23 weeks of age and continuing through egg laying, final incubation, hatching, and a 14-day offspring rearing period; the total approximate duration of treatment was 24 weeks. The birds were housed with one male and one female per pen, with 16 pens per dose group.

There were two mortalities in the control group and one each in the 25 and 125 ppm groups; these mortalities which were considered to be incidental to treatment as no treatment-related findings were seen at necropsy. No overt signs of toxicity were observed in any group. One female duck in the 125 ppm group had a prolapsed cloaca during week 13; after the prolapse was reduced, the duck appeared normal for the remainder of the study.

There were no significant differences in body weights, egg shell thickness, or offspring body weight between the control and any treatment group. At 500 ppm, feed consumption was slightly decreased (NS) throughout the study, with a significant difference from controls during weeks 6, 12, 14, and 19. There were no significant differences or apparent treatment-related effects in reproductive parameters at 25 and 125 ppm. However, while not significant, there appeared to be a slight reduction in the number of eggs laid by hens at 500 ppm during the last two weeks of egg production. During terminal necropsy, this reduction in egg production was correlated with an increase in the number of hens in the 500 ppm group with a regressing ovary.

Fish Full Life Cycle Toxicity (Fathead minnow, OCSPP 850.1500)

In a fish full life cycle toxicity study (**MRID 00102096, 1977**), groups of 60 fathead minnows (*Pimephales promelas*) embryos (60 embryos/aquarium; 2 aquaria/dose level) were exposed to permethrin (95.7% purity) in a flow-through system at nominal concentrations of 0 (control), 0, solvent control (7.2 mg/L DMSO), 0.063, 0.13, 0.25, 0.50, and 1.0 µg/L. During the initial 35 days of the chronic test, mean measured concentrations of permethrin were well below nominal concentrations and this trend increased as fish increased in size. To reverse the trend, nominal permethrin concentrations were increased to 0.094, 0.19, 0.38, 0.75, and 1.5 µg/L. The measured concentration upon which survival and percent hatch were compared to controls were 0-35 days: <0.023, <0.032, 0.092, 0.14, and 0.41 ppb; and 0-63 days: <0.042, 0.083, 0.17, 0.23, and 0.55 ppb. Percent hatch and survival was significantly different at the 0.41 ppb level. After 156 days exposure, surviving females were returned to spawning chambers in the ratio of 3 males to 7 females. The levels tested (measured concentrations) were <0.073, 0.16, 0.22, 0.32, and 0.87 ppb. At the 0.87 ppb level, there were no female survivors. At ≤0.32 ppb, survival, weight, length, and eggs/female did not differ significantly from controls. The second generation eggs were then exposed to mean measured concentrations of <0.11, 0.17, 0.30, 0.41 and 0.91 ppb. The % hatch for these levels did not differ significantly from controls but the survival of second generation fry at ≥0.41 ppb was significantly different than controls. To observe if there were residual effects of the chemical, fry were transferred from control groups to the 0.41 ppb test level and vice-versa; survival of 0.41 ppb fry transferred to control was significantly greater than those transferred to the 0.41 ppb test level.

Mean measured concentrations of 0.91 and 0.41 ppb significantly reduced the percent survival of fry during 30 days exposure. Minnows which survived the initial exposure period demonstrated normal ranges of measured parameters of survival, growth, reproduction, and egg hatchability. Based on the data, the researcher concluded the maximum acceptable concentration of permethrin for fathead minnows is estimated to be between 0.30 and 0.41 ppb. Residue analysis of fish showed bioaccumulation in female minnows as high as 4600x the water concentration. The majority of these residues were eliminated after 14 days in uncontaminated water.

Estrogenic Effects (Trout and Medaka)

In a published study by **Nillos *et al.* (2010),** permethrin stereoisomers were examined for estrogenic activity *in vitro* using transcriptional measurements of the egg-yolk precursor VTG in rainbow trout primary hepatocyte cultures, and *in vivo* using VTG protein measurements in male medaka. Additionally, the estrogenic activity of three permethrin metabolites [3-PBOH), 3-(4'-hydroxyphenoxy)benzyl alcohol (3,4'-PBOH), and 4-hydroxy permethrin (4-OH permethrin)] was investigated in the trout *in vitro* study.

For the *in vivo* VTG expression assay with medaka, adult male medaka were held at static conditions for 8 days with individual permethrin enantiomers, 1S-(+)-cis-, 1R-(−)-cis-,

1S-(+)-trans-, and 1R-(−)-trans-permethrin (>99% purity), at 10 µg/L, 17-β estradiol (positive control; concentration not specified), or 1% acetone (solvent control). Based on the reported results, the experiment included "control" and "mixture" test groups which were not defined; the study reviewers assumed that the control group included no chemical or solvent and that the mixture group was a mixture of all four permethrin enantiomers. On the last day of exposure, fish were anesthetized and the livers were excised and pooled for each individual replicate. The VTG protein level in the liver was determined using a commercial ELISA kit; the VTG concentration was normalized to the total protein in the liver homogenate. VTG production between the permethrin enantiomers was compared to determine enantio-selectivity.

For the *in vitro* VTG expression assay, trout hepatocytes were isolated from juvenile trout (confirmed to be without endogenous expression of VTG) and the hepatocytes were seeded in 48-well plates. Cells were incubated (18 °C for 24 h) with racemic permethrin at 50 µM, the individual permethrin enantiomers at 50 µM each, permethrin metabolites at 5, 10, 25, or 50 µM each, 17-β estradiol (positive control) at 0.036 µM, or 1% acetone (solvent control); the experiment included a control group and treatments were conducted in triplicate. The VTG mRNA expression in fish hepatocytes was measured using quantitative PCR after isolation of total mRNA from the cells using a commercial RNA extraction kit.

Microsomal biotransformation was investigated in livers pooled from five trout. The livers were homogenized and the microsomal fractions were resuspended. A reaction mixture of microsomal protein, substrate (racemate and enantiomers), NADPH, and Tris-HCl buffer was incubated. The supernatant was isolated by centrifugation for reverse-phase HPLC/UV analysis for metabolic products. Negative controls omitted the NADPH or included boiled microsomal protein. Cytochrome P450 inhibition tests were conducted by co-incubation with ketoconazole, which was added after addition of NADPH, and the mixture was incubated for 5 minutes prior to addition of the individual permethrin enantiomers.

After exposure of medaka *in vivo* for 8 days to the individual permethrin stereoisomers and permethrin mixture at 10 µg/L, hepatic VTG production was increased ($p<0.05$) compared to controls. Additionally, significant differences in the relative estrogenic potential of the enantiomers were reported: 1S-cis- and 1S-trans-permethrin had responses 2.5 and 1.3 times greater than their respective R enantiomers. The expression of VTG mRNA was significantly increased in trout primary hepatocytes exposed *in vitro* to the individual permethrin stereoisomers and permethrin mixture at 50 µM compared to controls. Additionally, a significant difference in the relative estrogenic potential of 1S-cis-permethrin and 1R-cis-permethrin was reported: 1S-cis-permethrin had ~2-fold higher VTG mRNA expression levels than 1R-cis-permethrin. A similar (but not significant) trend was observed for the trans-permethrin enantiomers.

The estrogenic activity of the permethrin metabolites (4-OH permethrin, 3-PBOH, and 3,4'-PBOH) was also investigated in the *in vitro* trout hepatocyte experiment. A concentration-related increase in the VTG mRNA expression level was observed in trout hepatocytes exposed to 4-OH permethrin and 3,4'-PBOH at 5, 10, 25, and 50 μM (the study authors did not state whether increases were statistically significant). For 3-PBOH, a significant increase in the VTG mRNA expression was only reported at 10 μM; the increase was not dose-dependent.

Stereoselective formation of three metabolites (4-OH permethrin, 3-PBOH, and 3,4'-PBOH) was observed following the incubation of permethrin in rainbow trout liver microsomes. Trans-permethrin underwent ester cleavage (resulting in formation of 3-PBOH and 3,4'-PBOH) more extensively than cis-permethrin. However, the predominant metabolite produced was the hydroxylated derivative of intact permethrin (4-OH permethrin). Furthermore, the cis-permethrin diastereomers appeared more susceptible to hydroxylation, yielding >2 times the amount of the hydroxylated product than the trans-permethrin diastereomers.

1S-cis-Permethrin had the highest NADPH-dependent metabolic conversion of the four permethrin stereoisomers, with the hydroxylated metabolite (4-OH permethrin) comprising 99% of the metabolite profile. Formation of 4-OH permethrin from 1S-cis-permethrin was ~25 times greater than the formation from 1R-cis-permethrin, demonstrating a significant stereoselectivity to 1S-cis-permethrin for the hydroxylation of permethrin. However, the NADPH-catalyzed cleavage of the cis-permethrin enantiomers was enantioselective for 1R-cis-permethrin.

Of all permethrin stereoisomers, 1S-trans-permethrin was found to be the most susceptible to ester cleavage. Although the trans-permethrin stereoisomers appeared more susceptible to ester cleavage than the cis-permethrin stereoisomers, hydroxylation in the fish liver microsomes was still higher than ester cleavage; 1S-trans-permethrin was more extensively hydroxylated than 1R-trans-permethrin.

Generally, ketoconazole significantly inhibited metabolite formation and enantiomer conversion when co-incubated with the individual enantiomers. The highest inhibition (98% reduction in conversion) was observed for 1S-cis-permethrin, likely due to diminished 4-OH permethrin formation.

The study authors concluded that the results of the *in vivo* and *in vitro* studies indicated stereoselective estrogenic activity of permethrin resulting from biotransformation of the parent compound to more estrogenic metabolites. 1S-cis-Permethrin was observed to have significantly higher activity than the 1R-cis enantiomer in both studies. All permethrin enantiomers were oxidized to the 4-OH permethrin metabolite and underwent esterase cleavage to 3-PBOH and 3,4'-PBOH. Racemic 4-OH permethrin as well as 3-PBOH and 3,4'-PBOH possessed significant estrogenicity.

Estrogen Activity in Fish

In a published *in vivo* study by **Brander *et al.* (2012**), evaluation of the concentration response to permethrin (99% purity; 50/50 mixture of isomers) with juvenile (65- to 70-day old) *Menidia beryllina* was examined in a 14-day static aqueous exposure bioassay. The biomarker selected was choriogenin (Chg), an estrogen-dependent egg coat protein, which was assessed with an indirect enzyme-linked immunosorbent assay (ELISA) relative to a positive control (ethinylestradiol). Fish were exposed to permethrin (0.1, 1, and 10 µg/L) or ethinylestradiol (1, 10, 50 ng/L) by adding stock solutions prepared in methanol (a control of 0.01% methanol was included). At study termination, fish were anesthetized (on ice), frozen, and homogenized in buffer, and the resultant supernatant after centrifugation was analyzed by ELISA. An *in vitro* chemical-activated luciferase gene expression (CALUX) bioassay was also conducted, using the human ovarian carcinoma (BG-1) cell line stably transfected with an estrogen-responsive luciferase reporter plasmid. The CALUX bioassay was used to determine the concentration-dependent agonist (pesticide alone) and antagonist (pesticide in presence of estradiol) effects of permethrin. Plated cells were incubated with carrier solvent (ethanol) and 17β estradiol (E_2; 1 nM), permethrin, or permethrin plus 1 nM E_2; permethrin was tested at 0.1-1,000 µg/L.

Mean survival in all *in vivo* treatments after 14 days was ≥80%, with the exception of 70% mean survival observed in the 10 µg/L permethrin group. Juvenile *M. beryllina* exposed to all three concentrations of permethrin and ethinylestradiol had higher ($p<0.05$) relative concentrations of Chg expressed in whole-body homogenates. None of the permethrin treatments were different from one another when compared with ANOVA (*p*>0.05); however, a trend toward an inverse correlation between response and increasing concentration with permethrin was apparent. Permethrin treatments were not significantly different from positive control concentrations of 1 and 10 ng/L; however, all permethrin concentrations trended toward a higher relative expression of Chg compared to the 1 ng/L ethinylestradiol treatment and a lower relative expression than the 10 ng/L ethinylestradiol treatment. All study concentrations of permethrin induced the expression of Chg in juvenile *M. beryllina* relative to control, and 1 ng/L permethrin induction yielded greater Chg expression compared to 1 ng/L ethinylestradiol.

For the CALUX assay, ER agonism was not observed for any of the permethrin test concentrations. For permethrin, some reduction in the ability of E_2 to induce ER-dependent reported gene activity was observed (~30-40% of maximal estradiol activity), but this inhibitory effect was not concentration-dependent (data presented graphically). It was concluded that permethrin was estrogenic in the *in vivo* test, but anti-estrogenic in cells *in vitro*, and noted that the difference observed between *in vivo* and *in vitro* results may be attributable to the lack of appropriate metabolism in the CALUX cell line.

Estrogen Effects (Embryo-Larval Zebrafish)

In a published study by **Y. Jin *et al*. (2009),** the estrogenic effects of permethrin enantiomers were evaluated by detecting the expression levels of estrogen-responsive genes in embryo-larval zebrafish. Permethrin (99.2%) was dissolved in acetone and diluted to 100, 250, 500, and 1000 ng/L in rearing water containing 0.1% acetone (v/v). The four permethrin enantiomers, (+)-cis-, (−)-cis-, (+)-trans-, and (−)-trans-permethrin, were also diluted to 250 or 1000 ng/L in water containing 0.1% acetone. Embryo-larval zebrafish were exposed to the above testing solutions under static renewal conditions for 7 days (0-7 days post-fertilization). Control embryos and larvae were exposed in water containing 0.1% acetone (v/v) only. The exposure solutions were renewed daily. No difference in survival rate was found during the study. Separate groups of embryo-larval zebrafish were exposed for 7 days to estradiol (E_2) at concentrations of 25, 50, 100, 250, 500, 1000 ng/L in water containing 0.1% acetone. Samples were homogenized and total RNA was isolated and reverse transcribed for real-time quantitative PCR analyses to determine the expression of vtg1, vtg2, esrα, cyp19a, and cyp19b genes.

The expression levels of vtg1, vtg2, esrα, cyp19a, and cyp19b mRNA in the zebrafish larvae were induced effectively when exposed to E_2 for 7 days. The lowest concentration of E_2 to significantly induce transcripts of these estrogenic-responsive genes was 50 ng/L, and the induction in mRNA levels of vtg1, esrα, cyp19a, and cyp19b were 5.0-, 2.6-, 2.3-, and 5.4-fold respectively, at this concentration compared with those in the control; vtg2 was the only one of these transcripts which was significantly induced at 25 ng/L E2. In addition, the E_2-induced increase in the mRNA level of the five genes was concentration-dependent. The sensitivity of these genes (in terms of the concentration needed to elicit a significant response) was the same, with the exception of vtg2 which was slightly more sensitive than the others. Thus, the expression of vtg1, vtg2, esrα, cyp19a, and cyp19b genes were adopted as biomarkers, and the 50 ng/L E_2 group was selected as the positive control in subsequent experiments.

Gene-specific responses to exposure to racemic permethrin were observed at concentrations ≥250 ng/L. The mRNA levels of three genes, vtg1, esrα, and cyp19b were higher ($p<0.05$) than those of the control group at exposure concentrations of 250, 500, and 1000 ng/L. In addition, permethrin induced the expression of the esrα in a concentration-dependent manner. Significant induction was observed for cyp19a only in the 500 and 1000 ng/L permethrin treatment groups. No significant difference was observed between the control and treatment groups for vtg2 expression, even at 1000 ng/L. Maximum induction in the transcript levels of vtg1, esrα, cyp19a, and cyp19b occurred in the highest exposure group, being about 3.8-, 5.0-, 4.8- and 3.0-fold higher than the control group, respectively. In the case of esrα and cyp19a, the induction folds in the highest permethrin treatment group were higher than that of the positive control group of 50 ng/L E2. From these results, embryo-larval expressions of vtg1, esrα, cyp19a, and cyp19b genes were adopted as biomarkers, and the 250 and 1000 ng/L racemic permethrin groups were selected as the positive control in the enantiomer exposure experiments.

Generally, at 250 ng/L each permethrin enantiomer exhibited a similar effect on the mRNA levels of the four selected genes as the racemate did. The only significant difference between enantiomers was found in cyp19b mRNA induction between the (+)-trans and (−)-trans enantiomer treatment groups. The mRNA levels of cyp19b in larval zebrafish receiving (−)-trans treatment were about 1.6-fold higher than those in the group treated with (+)-trans enantiomer. When the exposure concentration of permethrin enantiomers was increased to 1000 ng/L, the enantioselectivity in the induction of embryo-larval estrogenic-responsive gene expression became more obvious. The maximum induction in the mRNA levels of all four genes was observed in the group treated with (−)-trans enantiomer, being much higher even when compared with that of the racemate group. Moreover, a significant difference in the mRNA levels of vtg1, cyp19a, and cyp19b existed between the (+)-trans and (−)-trans enantiomer treatments. The mRNA levels of vtg1, cyp19a, and cyp19b of (−)-trans treatment were about 3.2-, 1.8- and 1.5-fold higher than that in the group treated with (+)-trans enantiomer, respectively. There was also a significant difference in mRNA levels of the cyp19b gene between the two cis-enantiomer treatment groups; the mRNA level of cyp19b in (+)-cis treatment group was about 1.5-fold higher than that in the group treated with (−)-cis-enantiomer. No significant difference was observed in expression of vtg1 or cyp19a between the two cis-enantiomers. For esrα, no significant difference was found between (+)-trans and (−)-trans or (+)-cis and (−)-cis-enantiomers, although the inductive tendency was similar to vtg1, cyp19a, and cyp19b. The study authors concluded that the results of these experiments strongly indicate the occurrence of significant enantioselectivity in estrogenic activity of permethrin enantiomers exposed to embryo-larval zebrafish

APPENDIX 3: References Not Utilized in the Permethrin WoE Analysis

In 2009, after public review and comment, a final list of 67 chemicals and schedule for issuing Test Orders for the EDSP Tier 1 screening battery was made available in a Federal Register Notice issued October 21, 2009 (74 FR 54422). The agency's review of the initial data submitted as "other scientifically relevant information (OSRI) was provided in the Report of the Endocrine Disruptor Review Team (USEPA, 2010).

Beginning in 2011, the agency has reviewed data cited as "OSRI which included Part 158 studies previously submitted to the agency for registration/reregistration, published literature articles and/or Tier 1 assays. The agency also conducted a more recent search (2009 to 2014) of available scientific literature for any additional relevant information for their weight of evidence (WoE) evaluations. These articles were evaluated in accordance with the agencies Evaluation Guidelines for Ecological Toxicity Data in Open Literature, May 2011 (http://www.epa.gov/pesticides/science/efed/policy_guidance/team_authors/endangered_species_reregistration_workgroup/PDF_rot/esa_evaluation_open_literature.pdf) and the 2012 Guidance for considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment (http://www.epa.gov/pesticides/science/lit-studies.pdf).

The following published and unpublished references were considered for use in the WoE analysis for Permethrin but were not utilized due to one or more of the following reasons: 1) the article was not available in English; 2) the compound of interest was not used in the study; 3) the test material was not adequately described; 4) a formulated end-use product or mixture of chemicals was utilized as the test material; 5) only acute mortality toxicity data were provided; 6) the experimental conditions were not adequately described; 7) only an abstract of the study was available; 8) the reference is a review article or book chapter and does not contain primary study data; 9) insufficient information was available to adequately assess the validity of the study results; 10) the 40 CFR Part 158 guideline study was classified as unacceptable/inadequate; 11) the study dealt only with non-EDSP assay development; 12) no specific endocrine-related endpoints were assessed in the study; and 13) the study contained only data on invertebrates.

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